UNIVERSITY OF ALBERTA

MOTOR COORDINATION
IN CONGENITALLY ACALLOSAL AND CALLOSOTOMIZED MICE

by

PETRA MELIKE SCHALOMON ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

DIVISION OF NEUROSCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Motor Coordination in Congenitally Acallosal and Callosotomized Mice* submitted by *Petra Melike Schalomon* in partial fulfilment of the requirements for the degree of *Doctor of Philosophy*.

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Dedication

I would like to dedicate this thesis to my parents, Dieter and Karin Schalomon of Bangalore, India. From an early age they impressed upon me the importance of education and the fulfilment to be found in learning. Because neither of them attended University they knew the regrets they felt at not having had the opportunity to fulfill their undoubted academic potential and they worked hard to provide their daughters with the means to get the education they wanted. Without their financial and, most importantly, moral support I would not be who I am today.

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Abstract

Behavioural consequences of transecting the corpus callosum (CC) and of congenital absence of the CC (ACC) were tested in mice. Transection of the CC in humans results in a syndrome with numerous deficits due to the lack of interhemispheric communication. Deficits in acallosal humans are less severe than those observed in CC transected patients, but selective deficits on bimanual coordination are found. The hypothesis that these deficits are due to the mechanisms of neural compensation employed by acallosal subjects was tested in large samples of recombinant inbred (RI) acallosal and hybrid CC transected mice. Methods for isoflurane anaesthesia and CC transection surgery in mice were developed. The CC was transected in a three cut approach which resulted in minimal extracallosal damage. In addition, cortical thickness was compared in normal, CC transected, and acallosal mice. CC status was not related to overall cortical thickness, but acallosal mice had thicker medial cortices. In Experiments 1 and 2, complex bimanual tasks were employed. In Experiment 1, measures of running on a wheel with missing rungs were monitored for five days. Hybrid mice performed better than RI mice, and mice with normal CC performed better than those with CC defects. Acallosal mice performed worse than all other groups and did not improve over time. In Experiment 2, mice had to traverse a notched balance beam. Findings were similar to those of Experiment 1. In Experiment 3, lengths of claws, which are cut by the animals with their incisiors, were measured. This constitutes an overlearned, unimanual task. Deficits were found only in RI mice, but normal RI mice improved with age, whereas acallosals did not. It is suggested that the route of compensation for the congenital

condition is an increased use of ipsilateral motor projections. The deficits on bilateral coordination in acallosal subjects may therefore be due to interfering motor commands from ipsilateral and contralateral motor pathways.

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List of Symbols and Abbreviations

AC	anterior commissure
ACC	agenesis of the corpus callosum
CC	corpus callosum
cin	cingular cortical thickness
cf	columns of fornix
CUD	crossed-uncrossed differences
dor	dorsal cortical thickness
F	fornix
F_2	second filial generation, second generation of inbreeding
H	Hippocampus
HC	hippocampal commissure
HRP	horseradish peroxidase
IP	intraperitoneal injection
IQ	intelligence quotient
lat	lateral cortical thickness
M	molar, molarity of a solution
med	medial cortical thickness
mg	milligram, 10^{-3} grams
mid	midway cortical thickness
min	minute
ml	millilitres, 10^{-3} litres

mm	millimetre, 10^{-3} metres
n	number of subjects
RI	recombinant inbred
rpm	rotations per minute
SD	standard deviation
α	alpha, probability of a Type I error on statistical tests
μm	micrometer, 10^{-6} metres
°	degree of angle
° C	degree Celsius

Chapter 1

INTRODUCTION

The corpus callosum (CC) is the largest fibre tract connecting the cerebral hemispheres in the brains of placental mammals, containing an estimated 200 million nerve fibres in humans (Zaidel, Aboitiz, and Clarke, 1995; Tomasch, 1954). It permits the transfer of information between primarily homotopic, but also between heterotopic, areas of the cerebral cortex (Lomber, Payne, and Rosenquist, 1994). The functions and development of the CC have been studied in samples of neurologically normal children of various ages (Roeltgen and Roeltgen, 1989).

The medical community first became interested in the CC when profound neuropsychological effects of surgical destruction of this structure were observed (e.g. Sperry, 1982; Sperry, Gazzaniga, and Bogen, 1969). Later it was shown that the functional consequences of congenital absence of the CC (referred to as ACC) differ significantly from those of surgical transection, being generally milder (Jeeves, 1977; Lassonde, Sauerwein, Chicoine, and Geoffroy, 1991). The two neurological phenomena, CC transection and ACC, thus permit insight into the different functional consequences of surgical disruption of a structure in the brain compared to agenesis during embryonal development.

It has been pointed out that the CC may be functionally heterogeneous, playing a primary role for some functions, that is, being necessary for specific tasks, and playing only a secondary role for others (Elberger, 1986). This heterogeneity might account for some of the divergent functional consequences of surgery as compared to ACC. Most likely, however, the differences in outcome of CC transection and ACC are due to the different compensatory mechanisms available to affected individuals. The main theories which have been evoked to account for the relative absence of functional deficits in ACC include a bilateral representation of functions in the acallosal brain (Ettlinger, Blakemore, Milner, and Wilson, 1972), the use of behavioural cross-cueing strategies (Sperry, 1968; Jeeves, 1977), growth of new, non-callosal fibres across the midline (Ettlinger, Blakemore, Milner, and Wilson, 1974), an increased use of already existing non-callosal commissural fibres, such as the anterior commissure, or the commissure of the superior colliculi (Ettlinger et al, 1974; Muente and Heinze, 1991), use of subcortical pathways (Milner, 1994), or an increased use of existing ipsilateral projections (Ettlinger et al, 1974).

To permit better insight into possible mechanisms of behavioural or anatomical compensation for CC defects, anatomical, etiological, and neuropsychological findings in CC transected patients and cases of ACC will be reviewed in the present chapter. This review will focus mainly on research investigating sensory-motor function in human patients, in whom the majority of behavioural research has been done, permitting a direct comparison of the two types of CC deficiency. Interestingly, little new research on this subject has been published in the years since 1994. During that year, a special issue of *Behavioural Brain Research* (Issue No. 64) focussing on the corpus callosum was published. In the same year, a comprehensive book of articles about callosal agenesis, edited by two of the most renowned researchers in this field, Lassonde and Jeeves, was published. Most researchers studying the function of the corpus callosum published research in one or both of these publications, and after the issue of these studies many appeared to feel that not much new remained to be done in this field for the present time.

Only with the development of new research approaches and techniques will this area of research become more active again in the future.

The present thesis describes at least one new technique which is useful in research on the function of the corpus callosum and on compensation for the loss of this structure. To date, no systematic experimental comparison of behavioural outcomes of CC transection and ACC has been possible in an animal model, because the congenital defect has been described in only one nonhuman placental mammal. Some strains of mice, most notably inbred substrains of BALB/c mice, exhibit ACC in a proportion of individuals (Wahlsten, 1989; Wahlsten, 1982; Ozaki, Murakami, Toyoshima, and Shimada, 1984). However, due to the relatively small size of the mouse brain, a surgical technique for CC transection has never been developed for this species. In order to enable researchers to use a cost-effective approach to research in the area of callosal deficiency, using group sizes that are sufficient to detect even small effects, a surgical technique for CC transection in the mouse which minimizes extracallosal damage is presented in Chapter 3 of the present thesis. Behavioural data on a number of measures of bimanual coordination in CC transected, acallosal, and normal mice could thus be collected and systematically compared in an attempt to find evidence for or against the use of specific compensatory mechanisms in the mouse brain.

1.1. Anatomy and Etiology

1.1.1. CC Transection

Early in the twentieth century, it was recognized that seizures in epileptic patients could spread from one hemisphere to the other via the CC, thus developing into generalized seizures. Beginning in 1939, the CC was surgically transected in patients in whom seizure activity could not be inhibited by drug treatments (Van Wagenen and Herren, 1940). Not until the classic research by Sperry (who was later awarded the Nobel Prize in Medicine for this work; Sperry, 1982) and his coworkers, was the effect of split brain surgery understood. This was partly due to the fact that the behavioural consequences of CC surgery are critically dependent on the exact location and extent of the actual transection. In some cases only the anterior part of the CC, the genu, is transected (e.g., Preilowski, 1977; Chen, Campbell, Marshall, and Zaidel, 1990; Jeeves, 1991), and in others only the central one third of the body of the CC is split in surgical treatments of intraventricular cysts or tumours located ventral to the CC (Bentin, Sahar, and Moscovitch, 1984). In extreme cases of epilepsy, the entire CC, including its most posterior part, the splenium, as well as other forebrain commissures such as the anterior commissure, are transected (Preilowski, 1977).

A number of other factors can influence the clinical outcome in CC transected patients. These include the length of recovery time after surgery and the age of the patient at the time of surgery. Temporary impairments in short term memory (Jinkins, 1991), in sequential organization of movements (Provinciali et al, 1990), and disruptive behaviour by the left hand (Tanaka, Iwasa, and Yoshida, 1990) have all been described during the first three to twelve months after callosotomy, after which these symptoms tend to disappear as neural reorganization and behavioural strategies may be put into effect. In addition, it has been suggested that in both animals and humans, CC transections do not interfere with interhemispheric communication if they are performed during a critical period of development (Lassonde, Ptito, and Lepore, 1990). In one experiment, kittens in which the CC had been cut after 45 days of age, but not kittens in whom this operation had been performed before 20 days of age, exhibited deficits in interhemispheric transfer (Lassonde et al, 1990). In humans, the CC is not fully mature until the age of 10 years (Yakolev and Lecours, 1967) and it is possible that before that age, sufficient neural plasticity exists to favour anatomical compensation. Lassonde, Sauerwein, Geoffroy, and Decarie (1986) documented a variety of deficits in a 16-year-old, all of which were significantly attenuated in patients who had undergone surgery at six to ten years of age. However, even these subjects, who had had surgery at young ages, had greater reaction times than older, normal controls. The authors suggested that these children most likely used ipsilateral connections coursing in extralemniscal spinothalamic pathways. Interestingly, Jeeves, Silver, and Milne (1988) found that normal six-year-olds, in whom the CC is relatively immature, also perform worse on a task requiring precise bimanual coordination than ten-year-olds or adults. Thus, young children also may be using ipsilateral connections for interhemispheric coordination of bilateral movements until the callosal connections are sufficiently mature for full transfer of sensory and motor information.

The above discussion illustrates that the behavioural outcome of CC transection cannot be represented as a homogeneous syndrome. Rather, it critically depends on the location and extent of surgery, with partial cuts resulting in subsets of classically described symptoms. In addition, the deficits are attenuated in patients undergoing surgery at a younger age and with increasing recovery time.

1.1.2. Callosal Agenesis

The prevalence of ACC in the general population is hard to estimate because it is a congenital problem and affected individuals may be almost asymptomatic (Bruyer, Dupuis, Ophoven, Rectem, and Reynaert, 1985). Estimates of the incidence range from between 3 to 7 out of 1000, being about ten times higher in developmentally disabled populations (Andermann, 1981). ACC may be complete, affecting the CC in its entire extent, partial, affecting only some callosal subdivisions, or of the hypoplastic type, which results in general thinning of the entire structure (Ettlinger et al, 1972; Andermann, 1981; Rauch and Jinkins, 1994a). In total agenesis, no fibres crossing the midline can be found, the columns of the fornix are separated in their entire extent, and the ventricles are enlarged. In addition, a large anomalous bundle of CC fibers, termed the Probst bundle, is found on either side of the midline in all cases of complete ACC except some associated with other severe neural anomalies (Stefanko and Schenk, 1977). This bundle has been interpreted as evidence of a misdirected growth of CC fibers after normal development is disrupted. In fact, ACC could be viewed as a failure of fibers to cross the midline rather than a true agenesis (failure to develop) of callosal axons (Stefanko and Schenk, 1977; Olavarria, Serra-Oller, Yee, and Van Sluyters, 1988). The underlying problem in ACC may thus be a lack of a suitable substrate for CC fibres to cross (Wahlsten and Ozaki, 1994). A study of embryonal development of the mouse CC has shown that in the normal brain, pioneer CC axons fasciculate along hippocampal commissure (HC) axons, which cross the midline earlier during embryonal development. In the brains of acallosal mouse strains, HC development is delayed beyond the time at which first crossing by CC axons occurs in normal brains, and thus the usual substrate for crossing of CC axons is missing, resulting in a later CC defect (Livy and Wahlsten, 1997).

Functional deficits in acallosals have not been studied as fully as those in split-brain patients, due to the high incidence of over 50 different anomalies associated with ACC and the concurrent low incidence of pure ACC (Wisniewski and Jeret, 1994). The most common symptomatology associated with ACC is mental retardation or developmental delay (Serur, Jeret, and Wisniewski, 1988; Lacey, 1985; Chiarello, 1980), although ACC in itself does not cause a decrease in IQ (Sauerwein, Nolin, and Lassonde, 1994). The neurological syndromes most consistently linked to ACC are Aicardi Syndrome (a triad of ACC, infantile epilepsy, and ocular abnormalities), Andermann Syndrome (sensorimotor neuropathy, retardation, and ACC), Shapiro Syndrome (spontaneous hypothermia and ACC), Acrocallosal Syndrome (a developmental field defect resulting in craniofacial malformations), and Menke's Disease (a disorder of copper metabolism resulting in kinky hair and ACC) (Wisniewski and Jeret, 1994), as well as Dandy-Walker Syndrome (cerebellar and ventricular malformations with ACC) (Golden, Rorke, and Bruce, 1987). A very high incidence of seizure disorders has also been reported; one researcher (Lacey, 1985) found more than 90% of patients diagnosed with ACC as infants developed seizures. In many cases these seizures seem to be restricted to one hemisphere (see for example, Chiarello, 1980), and indeed the interhemispheric coherence of electrical activity in a case of a human male with complete ACC was found to be decreased relative to what is found in the normal brain (Nagase, Terasaki, Okubo, Matsuura, and Toru, 1994). This correlation between epilepsy and ACC has also been confirmed in mice. Selective inbreeding of mice with partial BALB/c ancestry into two substrains with high and low seizure susceptibility actually resulted in one seizure resistant substrain with no CC defects and one substrain which was epilepsy prone and had a very high penetrance of the CC defect (Morin, Dolina, Robertson, and Ribak, 1994). However, it remains to be explored whether there is indeed a causal connection between ACC and seizure disorders .

As is the case for patients that have undergone CC transection, age of the patient suffering from ACC may play a role in the severity of behavioural deficits (Dennis, 1976). However, because ACC patients by definition are born with the disorder, age in this case also indicates the length of time during which neural or behavioural recovery could occur. Only one author failed to find changes in the extent or severity of deficits in a longitudinal study of two ACC patients from age 5 to age 20 (Jeeves, 1977). The behavioural tests used by Jeeves were quite complex, however, and imposed time constraints upon performance. Therefore, findings may have been due to the fact that tests were so difficult that even older acallosal subjects with less severe deficits than those exhibited by younger patients, were incapable of performing the tasks. Sauerwein, Lassonde, Cardu, and Geoffroy (1981), on the other hand, found deficits in performance speed on bimanual tasks which were more pronounced in younger patients. This finding would indicate that with age, compensation for the CC defect increases, resulting in a decrease in behavioural impairments.

Thus, as with CC transections, the pattern of deficits in ACC is far from homogeneous, primarily because pure ACC has rarely been described. In most patients, ACC is only one of a multitude of abnormalities (e.g. Serur et al, 1988), which very frequently include retardation and seizure disorders, making testing and the definition of appropriate behavioural norms difficult. Most studies therefore include epileptic or hydrocephalic control subjects with intact CC in addition to neurologically normal controls.

1.2. Deficits after Transection of the CC

In the early historical reports of surgical transection of the CC as a treatment of epilepsy, very few postsurgical deficits other than transient motor deficits, such as apraxia, were reported (van Wagenen and Herren, 1940). Even some more recent reports claim that there is no true disconnection syndrome (Mamelak, Barbaro, Walker, and Laxer, 1993), and explain this lack of disconnection symptoms after CC transection by the presence of abnormal cerebral organization in epileptic patients, with bilateral representation of language functions (Mamelak, Barbaro, Walker, and Laxer, 1994).

Many studies of split brain patients report low IQ scores (for example, Nagumo, Yamadori, Soma, Kayamori, and Ito, 1993; Strauss, Wada, and Hunter, 1994); however, IQ scores do not decline after the CC has been transected (Strauss, Wada, and Hunter, 1994; Mamelak et al, 1993). Low intellectual abilities of these patients must therefore be due to extracallosal defects of the forebrain, most likely as a result of the cerebral pathology, such as epilepsy, which made split brain surgery necessary. Other changes after split brain surgery, such as improvements in social behaviour (Provinciali et al, 1990) are most likely due to improvements in the epileptic condition of the patients after CC transection. The same may apply to improvements in memory documented in a number of patients with surgical sparing of the posterior CC (Provinciali et al, 1990), although others have found a decrease in both verbal and visual memory in some subjects with mixed cerebral dominance (Sass, Novelly, Spencer, and Spencer, 1988).

Most deficits commonly associated with CC split surgery involve language function, as described in the classic set of papers by Sperry and coworkers. While language comprehension appears to be unimpaired (Sperry et al, 1969; Gazzaniga, Kutas, Van Petten, and Fendrich, 1989), a language deficit can be observed when objects are presented tachistoscopically to only one visual hemifield, such that the information is processed in only one hemisphere. CC split patients were able to describe the stimuli as well as normal subjects when they were projected to the right hemifield, but reported that they had not seen anything when stimuli were projected to the left. When nonverbal responses were required of the patients, they performed well regardless of which visual hemifield the stimulus had been presented to (Sperry et al, 1969). Thus, the problem for the patients was not one of defective vision, but a defect in verbal output. The right hemisphere, which had received visual input, was not verbal and thus could not respond. The left hemisphere, which gave the verbal response, had not received the visual information, and thus indicated that nothing had been seen.

Pure somatosensory, tactual perception may be unaffected (Risse, Gates, Lund, Maxwell, and Rubens, 1989; McKeever, Sullivan, Ferguson, and Rayport, 1981). Tactile discrimination of textures between hands, by contrast, is less accurate and slower than in control subjects (Lassonde et al, 1986), and tactual learning and cross-localization of light (non-painful) touch between the hands is also somewhat impaired (Jeeves, 1977).

Motor performance, as well, appears to be affected by split-brain surgery. Provinciali et al (1990) reported transient ataxia and clumsy spatial sequential movements for up to three months after surgery. Irregular, heavy handwriting has also been observed in chronic split brain patients, indicating an impairment in the spatial organization of rhythmic motor patterns (Ten Houten, Seifer, and Siegel, 1988). Unimanual motor performance is affected even in chronic split-brain subjects with partial transections of the anterior CC (Chen et al, 1990). These patients have problems tying a knot using their right hand only and can not transfer proprioceptive information from one hand to the other without visual input when asked to imitate a posture imposed on one hand. With open eyes, the task is performed perfectly, as visual information is projected to both hemispheres and transfer is not necessary. Similarly, in monkeys, learned visuomotor responses were transferred successfully only if the anterior CC was spared in transections of both the CC and the anterior commissure (Eacott and Gaffan, 1990). In rats, CC sections were shown to disrupt a previously learned running pattern in a visual discrimination task (Christie and Steele Russell, 1990).

In addition to deficits of transfer of learned motor patterns, there is incomplete transfer of complex learned motor sequences between hands in human split brain patients (Bogen and Bogen, 1988). Because, however, there is only partial transfer of complex sequences in normals, it is unclear whether CC transected patients are actually impaired.

While bimanual motor performance was reported to be unimpaired in a sample of monkeys with transections of all forebrain commissures as well as division of the cerebellum (Elliott, Ettlinger, Maccabe, and Richardson, 1976), a wealth of data from human subjects indicates that, compared to other bimanual tasks, performance on bimanual motor tasks may be severely impaired after split brain surgery. The most common task used to assess bimanual motor coordination is one first described by Preilowski (1977). It employs an apparatus with a pencil whose motion can be controlled by two crank handles, each of which is operated with one hand. One handle controls vertical, the other horizontal displacement of the pencil on a piece of paper. The patient's task consists of drawing a line along a narrow horizontal, vertical, or diagonal track on the paper by turning the crank handles. For this task, both hands have to move simultaneously, the relative output of each hand being determined by the slope of the track. Preilowski (1977) reported that patients with complete commissurotomies were unable to perform the task, and that two patients with anterior CC transections, who initially were able to perform as well as controls, showed no improvement with practice and required twice as much time to complete the task as controls in later trials. Similar apparatuses were used by a number of other researchers (Jeeves, Silver, and Jacobson, 1988), who reported that an 11-year-old patient with a lesion of the central third of the CC was able to perform as well as controls on this task, both with respect to speed and to accuracy. However, it can be assumed that this patient's good performance was a result of his age, and the resulting capacity for plasticity of function, as well as limited size of lesion, all of which can mitigate behavioural deficits (Jeeves, Silver, and Jacobson, 1988; Bentin, Sahar, and Moscovitch, 1984).

An alternative explanation for bimanual deficits in split brain patients has been suggested by research on the alien hand syndrome. According to Geschwind, Iacoboni, Mega, Zaidel, Cloughesy, and Geschwind (1995), the archetypal alien hand syndrome is characterised mainly by oppositional intermanual conflict, such that the non-dominant hand interferes with performance of the dominant hand. One patient with lesions to the body and genu of the CC (Jason and Pajurkova, 1992) exhibited a lack of cooperation of the left, non-dominant hand, which could actually be predicted by the patient before the movement was begun. The authors concluded that in this case, the defect was not one of coordination, but of cooperation. However, this syndrome is usually transient, and may be due to acute effects of surgical retraction of the cerebral hemispheres (Bogen, 1993, Sauerwein and Lassonde, 1994). In one patient with infarction of the body and genu of the corpus callosum tested years after the onset of callosal pathology, right hand use actually facilitated performance by the non-dominant left hand (Nagumo et al, 1993). It is therefore unlikely that all bimanual deficits in split brain patients can be ascribed to a lack of intermanual cooperation.

Another consequence of transecting the CC appears to be a deficit in visual abilities. McKeever et al (1981) reported intact transfer of visual information after transections of the anterior half of the CC, which was eliminated after complete transection. More precisely, visual transfer was shown to be intact after surgeries sparing as little as the posterior third to fifth of the CC as well as the splenium (Risse et al, 1989), indicating that only the caudalmost portion of the CC is necessary for visual transfer.

A more intriguing topic is that of central vision, in which perception of stimuli directly behind or in front of the point of fixation is tested. Mitchell and Blakemore (1970) proposed inputs from the ipsilateral hemifield of vision to binocular units in the cortex, which are maximally responsive to stimuli behind or in front of the fixation point. If this input were mediated via the CC, then split-brain patients would be unable to judge whether a stimulus was closer or further away than the point of fixation. This hypothesis was confirmed by Mitchell and Blakemore (1970) whose results have since been replicated by Jeeves (1991).

It is now obvious that a number of deficits in interhemispheric transfer can be found in split brain patients. The most apparent deficit involves the lack of transfer of language from the left to the right hemisphere in patients with complete CC transections or sections of the anterior CC. In addition, the transfer of somatosensory and motor information is impaired by lesions in the anterior part and body of the CC. Nevertheless, many of the deficits on tactile and motor tasks are transient or partial, indicating that some behavioural recovery is possible; this may be mediated by remaining callosal fibres as well as cross-cueing strategies.

1.3. Deficits in Callosal Agenesis

While ACC patients may be of normal intelligence (Bruyer et al, 1985; Gott and Saul, 1978; Jeeves and Temple, 1987), a frequent deficit is low intelligence with both low performance IQ and low verbal IQ scores (Field, Ashton, and White, 1978). General memory performance in total ACC may be slightly impaired (Ettlinger et al, 1974, Bruyer et al, 1985), with minimal memory deficits in cases of partial ACC (Geffen, Forrester, Jones, and Simpson, 1994). Others have concluded that memory in cases of callosal damage is impaired only if there is also extracallosal damage (Clark and Geffen, 1989), however, the latest research would indicate that memory problems in ACC are due to deficits in memory consolidation resulting from the CC abnormality (Geffen, Forrester, Jones, and Simpson, 1994).

The possibility of memory deficits must be taken into account in examining the performance of acallosal subjects, as memory problems could affect performance on other tasks that have memory components. IQ matched control subjects are thus generally used; however, even this methodology could lead to false conclusions because low IQ control subjects usually do not have comparable neurological pathology in areas other than the CC (Cook, Brugger, Regard, and Landis, 1990).

In contrast to split brain patients, acallosals apparently are able to access language function with either hand (Lassonde et al, 1991), although there has been at least one report of random naming of objects held in the left hand (Koeda and Takeshita, 1993). In addition, recent research has shown that there are subtle language deficits which may be more pronounced in females (Jeeves and Temple, 1987), and include deficits on auditory discrimination and repetition of non-words (Temple and Ilsley, 1993), rhyme retrieval (Jeeves and Temple, 1987), as well as the production and recognition of rhyme (Jeeves, 1992). Some of these deficits may be due to the fact that acallosal patients have difficulty in localizing sounds in space, particularly when speed or precision of the response is required (Poirier, Miljours, Lassonde, and Lepore, 1993). The lack of deficits on most other language functions could be due to either interhemispheric transfer or to the development of bilateral language capabilities in ACC, a theory for which there is very little supporting evidence (Chiarello, 1980; Sauerwein et al, 1994).

Pure tactile performance was also tested by tasks which required intermanual comparisons between stimuli of different shapes, sizes, and textures. These tasks have few or no motor components and have revealed no deficits of acallosal subjects (Lassonde, Sauerwein, McCabe, Laurencelle, and Geoffroy, 1988; Reynolds and Jeeves, 1977; Ettlinger et al, 1974). However, a reduced speed of performance was observed (Lassonde et al, 1988). In addition, a diffusely distributed, lowered threshold for detecting pressure was observed in one patient after tactile deprivation in a circumscribed area of the forearm (Dennis, 1976). This observation may constitute evidence for non-topographic coding of tactile information in ACC, a conclusion that will have to be confirmed in further research.

Tactile perception was shown to be unimpaired in ACC in a test that involves visual tachistoscopic presentation of pictures of objects, which acallosals had to retrieve by touch alone (Saul and Sperry, 1968). When asked to identify tachistoscopically presented objects verbally, acallosals also perform like controls (Ettlinger et al, 1974).

However, when asked to compare colours presented to the two visual hemifields on a similar task, acallosals do show an impairment. The extent of this impairment has been variously described as complete, resulting in scores close to chance level (Ettlinger et al, 1974) or as very mild, with error rates of six percent (Lassonde et al, 1988). These results were obtained using dissimilar procedures, however. Ettlinger et al (1974) required their subjects to identify drawings of different objects, words, or even to judge similarity of slightly phase-shifted wave patterns which were presented for 100 ms. The task employed by Lassonde et al (1988) was less complex and merely required same-different judgements of simple geometric forms and colours, which were presented for 150 ms. Error rates of control subjects, too, were higher in the study by Ettlinger et al (1974), which indicates that the task employed by Ettlinger was indeed more difficult. As a consequence, his results may well have been due to the increased task demands. This conclusion is further supported by the findings in one ACC patient, whose accuracy on comparisons of visual stimuli presented tachistoscopically to the visual hemifields depended on the similarity of the visual stimuli that had to be compared, dropping to chance levels if a detailed analysis of the visual material was required (Karnath, Schumacher, and Wallesch, 1991).

In tests of stereoscopic depth perception, acallosals performed normally when peripheral depth perception was assessed (Ettlinger et al, 1974). When tested with stimuli behind or in front of the fixation point, on the other hand, acallosals, like split-brain patients, performed at chance levels (Ettlinger et al, 1974; Lassonde, 1986). Acallosals thus may not have developed compensatory mechanisms for the lack of callosal input to binocular cells which are responsive to midline stimuli.

The association of ACC with clumsiness has often been described and performance scores on standard IQ tests may be significantly below verbal scores (Bigler, Rosenstein, Roman, and Nussbaum, 1988; Gott and Saul, 1978). In addition, when being compared to non-ACC subjects with comparably low IQ scores, ACC subjects are generally inferior on sensorimotor learning tasks (Sauerwein et al, 1994). By contrast, some ACC subjects show almost normal, though unusually slow, motor performance on fine motor tasks such as stringing beads or wrapping a string around a pencil (Field et al, 1978; Reynolds and Jeeves, 1977).

Many of the motor deficits associated with ACC involve the dominant hand. Jeeves and Silver (1988) reported a case of ACC with deficits of grasping movements. The task was one of reaching for a horizontally suspended saucer and grasping it with the dominant hand. Normal control subjects simultaneously stretched their arms and began to close their fingers and thumb such that the saucer was firmly grasped upon contact. The ACC subject, by contrast, reached out until contacting the saucer and then began to close his fingers. Others have also found a deficit in the speed of grip formation, which was most profound when reaches into the contralateral hemispace were required by either hand, mostly due to the fact that acallosals spent more time decelerating in anticipation of the grip (Jacobson, Servos, Goodale, and Lassonde, 1994). This behaviour resembles that of infants (Von Hofsten, 1979), and may be evidence for an enhanced use of ipsilateral pathways in ACC similar to that observed in children before maturation of the CC.

Deficits on fine motor coordination are also seen in the nondominant hand. Jeeves (1977) reported very significant deficits in writing with the non-preferred hand by two acallosals, which did not improve from age 5 to 20 years. In addition, Dennis (1976) reported involuntary movements of adjacent fingers which accompanied voluntary movement of specific fingers. This finding indicates a definite problem with response differentiation of fine distal movements in cases of ACC.

It is therefore not surprising that motor deficits may be highest on tasks with a spatial component, such as tactile mazes or formboard learning, which require precise relative localization of movements (Chiarello, 1980). Whether transfer of learning from one hand to the other on tasks such as tactile mazes or formboards is possible in acallosal patients is debatable. Two siblings (ages 9 and 17) with total ACC demonstrated no deficits of transfer of formboard learning from the dominant to the nondominant hand (Sauerwein et al, 1981). It has been suggested that deficits in the transfer of formboard learning may be due to the use of inadequate behavioural strategies for performance of the task. Acallosal patients who learned either the location of specific holes on the formboard or who visually matched shapes to the form of the holes were successful at transferring learning, while one patient who employed a trial and error strategy in solving the task did not show transfer (Imamura, Yamadori, Shiga, Sahara, and Abiko, 1994). Similarly, Jeeves (1977) documented transfer of learning on a tactual pencil maze in two completely acallosal, adult subjects. By contrast, others (Gott and Saul, 1978; Reynolds and Jeeves, 1977) found no evidence of transfer of learning on a stylus maze and formboard task in completely acallosal adults. It is interesting to note that Reynolds and Jeeves (1977) did find significant transfer of finger maze learning when the task was repeated using a larger, less complex maze. Thus, the inconsistent findings in the literature may be due to differences in the relative demands on each task, with a breakdown of transfer when the information is more complex.

Not surprisingly, acallosal subjects are also impaired on bimanual motor tasks. In mice, Lipp and Wahlsten (1992) have reported a clear relationship between a decrease in CC area in various strains of acallosal mice and an increase in errors on a task requiring hindlimb coordination. However, there were large individual variations, and it appeared that rather than causing a direct deficit in coordination, the CC defect resulted in an increased likelihood of the coordination deficit. In humans, an inability to button clothes or buckle a belt have been reported (Field et al, 1978) as well as clumsy and slow performance on tasks requiring the stringing of beads (Field et al, 1978; Jeeves, 1977) and tying of shoes (Sauerwein et al, 1981) in a number of children and adolescents with ACC. Again, individual performance on these tasks was highly variable and it therefore appears that some patients can compensate for their hereditary deficits.

The classic bimanual task developed by Preilowski (1977), which involves the turning of two crank handles to draw diagonal lines with a plotter, has also been used to assess cases of ACC. Jeeves, Silver, and Jacobson (1988) found that three acallosals showed various degrees of impairment on these tasks, but none of them was able to match the performance of control subjects with respect to either accuracy or speed. In an extension of this research, Jeeves, Silver, and Milne (1988) were able to show that with practice, normal subjects no longer rely on visual feedback to perform Preilowski's (1977) task, and their performance can actually be inhibited by visual feedback. Acallosals, on the other hand, never lose their reliance on visual feedback for this task and their performance deteriorates significantly when visual feedback is blocked. These results again indicate that some amount of transfer of motor information does exist in most ACC patients; yet the alternate pathway for information transfer is inefficient (and actually comparable to that of normal six-year-old subjects; Jeeves, Silver, and Milne, 1988) and does not permit improvement beyond a certain level of performance. In addition, there is evidence that brains of ACC patients may not be normally lateralized, because these patients have particular problems drawing lines at angles of 112.5 degrees, which require less movement with the right as compared to the left hand (Silver and Jeeves, 1994). Drawings of lines at angles which required movement by either hand alone (90 or 180 degrees), by both hands equally (135 degrees), or which required more movement by the dominant, right, hand than by the non-dominant left hand (157.5 degrees), were significantly better executed.

Animal research, too, has indicated effects of ACC on cerebral lateralization. While some researchers have reported a decrease in paw preference (the animal equivalent of handedness) in male I/LnJ mice, which are highly inbred and never have a CC (Gruber, Waanders, Collins, Wolfer, and Lipp, 1991), others have found no relationship between the size of the CC and magnitude of paw preference in BALB/cCF mice, an inbred mouse strain in which typically fewer than 20 percent of all individuals have a small or absent CC (Bulman-Fleming, Wainwright, and Collins, 1992). BALB mice, in contrast to Swiss mice, which have normal callosa, showed a populational preference for left paw use (Schmidt, Manhaes, and de Moraes, 1991). Another indication of CC effects on lateralization came from a study by Schmidt and Lent (1991), who found that 75% of BALB mice with CC defects exhibited rotatory behaviour to the left, whereas normal

BALB mice showed no populational preference for direction of rotation. By contrast, no differences in asymmetries on tests of early reflex behaviour were found between BALB mice with normal or defective CC (Laroche and Ward, 1992), a finding that indicates that the CC does not affect the degree of asymmetry on at least some tests of sensorimotor behaviour.

The conclusion that can be reached from the above evidence is that the most obvious deficit in the performance of acallosals appears to involve speed and efficiency of performance. Even if subjects are capable of completing a given motor task, they often do so only clumsily or slowly (e.g. Ettlinger et al, 1972; Jeeves, Silver, and Jacobson, 1988; Sauerwein et al, 1981). This may be the result of a reduced efficiency of alternate pathways for the transfer of information between the hemispheres or of a lack of inhibition of ipsilateral input due to absence of callosal fibres.

This reduced efficiency of transfer is also evident on a number of non-motor tasks, such as intermanual or visual matching. There is little evidence of deficits on language-related tasks, by contrast, and performance on tasks involving visual midline stimuli is very low regardless of task demands.

1.4. Compensatory Mechanisms

It is evident that both split-brain patients and acallosals have some deficits on tasks involving interhemispheric transfer of information. The extent and quality of these deficits are not homogeneous between or within these two groups, however. Generally, more and stronger deficits are found in patients with complete transection of the CC as compared to those with incomplete transection or acallosals.

Deficits in split-brain patients are found on a wide range of tasks, and the exchange of information between the cerebral hemispheres in patients with complete CC transections appears to be inhibited (Sperry et al, 1969). Symptoms are attenuated only in cases of incomplete CC transection, or in very young patients in whom significant neural plasticity or learned compensatory behaviours may enable functional recovery. In adult surgical patients, in whom there is comparatively little neural plasticity, deficits are most severe.

Deficits in acallosal patients may be less severe and do not affect as many different functions. Generally speaking, functional deficits in ACC are greater in younger patients; presumably due to the fact that the brain is immature and neural recovery is incomplete and due to a lesser degree of learned compensation. Affected patients with more severe neurological abnormalities also have a reduced life expectancy (Carpenter, 1994; Geoffroy, 1994), and thus will rarely be included in older samples.

A number of possible functional mechanisms has been proposed to account for the pattern of deficits as well as preservation of functions in the split brain patient. It is evident that in adult callosotomized patients no callosal transfer of information exists and that little neural compensation can occur. Many authors imply that much of the recovery of split brain patients is due to complex cross-cueing strategies (e.g. Sperry, 1968; Reeves, 1991), though this theory has not been explicitly tested. Some lack of deficits in partial commissurotomy patients may also be due to the fact that the CC fibres carrying that specific information have not been transected. Some researchers (Jeeves, Silver, and Jacobson, 1988) propose an inhibitory function of the CC on motor control in the normal brain. When the CC is cut, inappropriate responses controlled by ipsilateral projections may no longer be inhibited, resulting in an exacerbation of deficits. Most evidence, however, points to an excitatory function of the CC in normal brains (Lassonde, 1986; Clarke and Zaidel, 1994), which can become inhibitory only when interneurons relay information from the CC to target areas (Berlucchi, 1990). It is possible that ipsilateral projections degenerate due to lack of use during the normal course of brain development in childhood, and thus usually before surgery, in split brain patients (Jeeves, 1994).

To account for the relative absence of deficits in ACC patients, four main theories of compensation have been proposed. These include the possibility of bilateral representation of functions, the use of behavioural cross-cueing strategies, an increase in the use of extracallosal commissures, and the use of ipsilateral projections. The first of these four theories indicates that some functions in the acallosal brain may be bilaterally represented, which would decrease the need for interhemispheric communication (Ettlinger et al, 1972). Evidence has mounted against this position (Ettlinger et al, 1974; Muente and Heinze, 1991), and recent evidence on abilities of acallosals to compare stimuli delivered simultaneously to the hemispheres cannot be accounted for by bilateral representation of functions (Lassonde et al, 1990). The possibility of bilateral representation of at least some somesthetic function has not yet been eliminated, however (Lassonde, 1994).

Only one of the proposed compensatory mechanisms is purely behavioural and relates to cross-cueing strategies. In a test of reaction times of motor responses to visual stimuli, three acallosals were shown to lie above the normal adult range of reaction times for crossed/uncrossed differences (CUD, the difference in response time between intrahemispheric and interhemispheric responses) on distal responses (Aglioti, Berlucchi, Pallini, Rossi, and Tassinari, 1993), and were comparable to the performance of children in whom the CC was functionally immature (Brizzolara, Ferretti, Brovedani, Casalini, and Sbrana, 1994). Nevertheless, reaction times were too short to be accounted for by cross-cueing strategies. The most cogent argument against the use of behavioural strategies, however, is the question of ecological value, as a need for complex strategies for dealing with unilateral visual stimulation and the like rarely exists outside a laboratory setting. In everyday life few visual stimuli of importance are present only for very short periods of time in the most lateral aspects of the field of vision. If any stimulus of interest is ever detected in such a position, the most efficient way of making the information available to both hemispheres would be to turn the head toward the stimulus (and thus project the image to both visual hemifields) rather than to employ complex compensatory strategies. Thus it is unlikely that such strategies would ever be developed by acallosal subjects.

One remaining hypothesis for compensatory mechanisms in ACC is concerned with alternate routes of information transfer between the hemispheres. New, non-callosal fibres may grow across the midline in cases of ACC (Ettlinger et al, 1974). In addition, already existing non callosal commissural fibres, such as the anterior commissure (AC) or the commissure of the superior colliculi, may be increasingly used or developed (Ettlinger et al, 1974; Muente and Heinze, 1991). Little concrete evidence for an increase in size of non callosal commissures has been found, however (Stefanko and Schenk, 1977; Fischer, Ryan, and Dobyns, 1992). A rerouting of CC fibres through the AC in mice (Wahlsten and Ozaki, 1994), or an increase in total number of myelinated AC fibres in acallosal mice have not been found either (Livy, Schalomon, Roy, Zacharias, Pimenta, Lent, and Wahlsten, 1997). On the other hand, an 18% increase in the number of unmyelinated axons has been found in the AC of acallosal mice (Livy, Schalomon, Roy, Zacharias, Pimenta, Lent, and Wahlsten, 1997). In addition, anatomical evidence shows that AC fibres, in contrast to splenial fibres, do not project to the entire temporal isocortex in monkeys. In fact, AC and CC fibres project to separate territories in those cortical areas that both commissures subserve (Zeki, 1972). Lent and Guimaraes (1990) found that AC axons in the hamster brain project mainly to paleocortical areas, such as pyriform, insular, perirhinal, and entorhinal cortices, as well as to temporal isocortex.

Recently, several researchers have speculated that only specific types of information may be transferred interhemispherically via non-CC commissures (Jeeves, 1992). Lassonde (1994) stated that visual information in the acallosal brain is most likely rerouted via the AC or subcortical fibres and while evidence does not actually support a role of the AC in intact bilateral visual integration, there is evidence for the use of the intertectal commissure (Milner, 1994).

The last proposed compensatory route is an increased use of existing ipsilateral projections such as auditory and extralemniscal sensory pathways (Ettlinger et al, 1974). Whereas in callosotomized individuals these projections may no longer exist at the time of surgery (Jeeves, 1994), they are abundant in the immature brain and in cases of ACC may not regress or may undergo an extended period of plasticity (Jeeves, 1994; Jeeves, 1992; Windrem, de Beur, and Finlay, 1988). This mechanism would also account for the fact that ACC patients show deficits in speed and precision of performance on many tasks due to the fact that more time may be required for conduction of the neural impulse via this pathway. In addition, whereas some have urged caution in assuming that the normal CC may inhibit ipsilateral pathways, it has long been argued that in ACC, ipsilateral and contralateral motor projections compete, resulting in impaired coordination due to poor coding of stimulus topography (Dennis, 1976; Jeeves, 1992).

The most likely explanation for the observed pattern of deficits in ACC is that several of the above compensatory mechanisms are used. Whereas visual, auditory, and language information may be transferred via the AC and subcortical pathways, at least one expert has argued that there is no non-CC mechanism for interhemispheric transfer between frontal and posterior parietal areas, which are involved in motor and spatial control (Lassonde, 1994). This would lead to the use of ipsilateral projections for motor coordination and a consequent impairment on highly complex or speeded tasks. In callosotomized individuals there is no evidence for the use of ipsilateral projections, therefore a similar impairment pattern would not exist.

1.5. Techniques and Experimental Design

There is some experimental evidence for greater deficits of acallosal patients on bimanual coordination tasks in spite of the fact that callosotomized patients generally have larger deficits than ACC patients (see above). However, in the past, testing has generally been performed on small samples of human acallosal patients, in case studies rather than scientific experimentation . One reason for the lack of research utilizing an animal model, which permits better experimental control and the use of large sample sizes has been the lack of a surgical method for callosotomy in the mouse, the only nonhuman species which exhibits callosal agenesis, and thus the only nonhuman species in which systematic testing of acallosal and callosotomized subjects could be undertaken. Callosotomy surgery in small rodents published in the past has always been performed on rats, and always resulted in extensive extracallosal damage to cortex, hippocampus or the cerebellum (see for example, Noonan and Axelrod, 1991; Crowne, Novotny, and Russell, 1991; Goodman and Russell, 1974), making results of behavioural testing difficult if not impossible to interpret. The surgical method presented in Chapter 2 of this thesis minimized extracallosal damage, and thus permitted the collection of meaningful behavioural data in callosotomized mice. Unfortunately, the additional improvement of using isoflurane gas anaesthesia to decrease fatality rates due to complications with anaesthesia which is described in Appendix 1, was not developed until after an article on the surgical method had been written and published, and thus has not been included in Chapter 2, which was based on the published article. Subjects used in the behavioural testing described in Chapter 4, however, underwent callosotomy or sham surgery while under isoflurane anaesthesia.

The development of these methodological advances thus permitted systematic testing of bimanual coordination under conditions of high task demands, in sufficiently large samples of acallosal and callosotomized mice, which can be exposed to more rigorous experimental control than would be the case with human subjects. This research finally gives insight into causal relationships of patterns of neurological deficits and behavioural abnormalities. If control in fine bimanual tasks in acallosal mice does in fact involve an increased use of ipsilateral fibres, their performance would be worse than that of CC transected mice, who presumably use unilateral motor and sensory information without interhemispheric integration. If, on the other hand, acallosal mice relied on crossed pathways such as the AC or subcortical fibres, their fine motor control would be superior to that of CC transected mice, because in that case there would be interhemispheric integration of sensorimotor information in the acallosals. If both these processes were simultaneously active in acallosals, performance levels similar to those observed in split brain mice would result.

These hypotheses were tested by examining acallosal, CC transected, sham operated, and normal mice on a battery of motor tests requiring fine distal control. As deficits in human acallosals are clearly exacerbated under conditions of high task demands, the tasks involved testing the animals at their limits of motor output both with regard to speed and precision of movements. In addition, some of the strains of genetically acallosal mice used in this study showed incomplete penetrance of the defect, such that various animals of the same strain showed complete or partial agenesis or normal development of the CC. The normal mice of the acallosal strains thus served as controls for strain differences, whereas the mice with partial ACC helped elucidate the relationship between CC size and magnitude of any defect in coordination.

An important point to remember with respect to the present body of research is that while the various genetic backgrounds of subjects used were probably important with respect to performance on behavioural tasks, it was not the focus of this research. The behavioural research described in Chapter 4 was specifically undertaken in an attempt to understand the functional consequences of the loss of the corpus callosum in mice, which so far has been completely unknown. This meant that subjects with both congenital and acquired absences of callosal connections had to be examined. From research with human patients suffering from ACC, it is apparent that congenitally acallosal subjects do not show deficits on behavioural tasks that are insufficiently complex. Therefore, if the present research had been done using only acallosal mice and had failed to discover any behavioural deficits, the lack of deficits on the task could have been due to one of two distinct reasons. The task used could have been insufficiently complex and therefore insensitive to congenital CC defects, or alternatively the CC in mice might not be essential to performing bimanual motor coordination tasks. By using callosotomized subjects in addition to acallosal subjects, the only viable explanation for a lack of deficits on any task would be that the task did not require a normal CC.

Thus, what is described in this thesis is actually a series of parallel experiments, testing bimanual motor performance in acallosal subjects *and* in callosotomized subjects. It does not constitute a true comparison of behavioural deficits in acallosal *versus* callosotomized subjects because the genetic background of callosotomized subjects (which were hybrid mice of the B6D2F_2 strain derived from the inbred strains C57BL/6J and DBA/2J) always differed systematically from the genetic background of acallosal subjects (which were recombinant inbred (RI) mice derived from the inbred strains 129/ReJ and

BALB/cWah1). Control subjects used were also from both the $B6D2F_2$ hybrid strain and from several RI strains, such that callosotomized and acallosal mice could always be compared to control mice with comparable genetic background. Therefore, in the design of the present research, genetic background of subjects and mechanism of callosal defect were not fully crossed: the group of subjects in each of the two different genetic backgrounds could only be affected by one of the mechanisms of callosal defect, not by either one.

In addition, acallosal mice did not all share the same genetic background. Although they had all been derived from the same cross of 129/ReJ and BALB/cWah1 mice, a number of separate strains had been established and maintained by full-sibling inbreeding in the second generation of inbreeding from the original cross. As a result, the RI lines used in this research differed in terms of genetic background and in terms of CC anomaly, because some of the strains used showed complete absence of the CC, some were always normal with respect to CC development, and some showed incomplete penetrance of the defect. Therefore, if RI mice with different degrees of CC pathology also differed in behaviour, no causal pathway of genetic background (absence versus presence of a mutation causing ACC), callosal abnormality, and behavioural deficits can be proven with the present experimental design. Rather, two separate models could be supported by such data. According to Model 1, differences in genetic backgrounds cause differences in CC development, and these differences in CC pathology directly cause differences in behaviour. According to Model 2, differences in genetic backgrounds cause differences in CC development, and simultaneously cause differences in behaviour.

Thus, CC pathology and behavioural differences would still be indirectly related, but no causal connection would exist between them.

To prove a causal pathway of genetic background, CC abnormality, and behaviour would require callosotomy surgery in an RI strain with incomplete penetrance of the CC defect. At present, these strains are not yet highly inbred, and therefore some substrains include mice with completely normal CC, and others with complete or partial absence of the CC. After performing surgery in these strains, mice from the same genetic strain, some with a normal CC, some with ACC, and some with a surgically transected, but previously normal, CC could be obtained. Then, if Model 1 was correct, both groups of mice with CC pathology, whether congenital or acquired, should exhibit similar behavioural deficits, though maybe to differing degrees, due to differences in the time of onset of the defect. If, on the other hand, Model 2 was correct, only the mice with the genetic mutation causing ACC should exhibit the behavioural deficits associated with ACC. Mice with callosotomies might exhibit behavioural deficits, but these would be due to completely different underlying mechanisms and should differ not only quantitatively, but also qualitatively from those seen in acallosal subjects.

There is a simple reason why this true comparison of the behavioural effects of ACC *versus* callosotomy is not presented in the present document. Limitations in terms of time as well as funding make it impossible to complete research projects which leave nothing more to be explored. It was important to perform callosotomy in neurologically normal hybrid animals, in order to see the effects of pure callosal transection in the absence of any other abnormality. If only RI mice with callosotomies had been used, any behavioural abnormality observed in callosotomized subjects could have been due to the interaction of surgery and the RI genetic background. The use and subsequent comparison of callosotomized subjects of both RI and hybrid backgrounds would have been ideal, but was not feasible. It, along with a number of other avenues of research, such as callosotomy in infants or fetuses, which would match the time of onset of the CC defect between ACC and callosotomy, must be left for future research. Other experiments, like a comparison of the effects of ACC in hybrid versus RI subjects, which would be necessary in an ideal world of fully crossed factors of genetic background and type of CC defect, obviously can never be done.

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Chapter 2

A PRECISION SURGICAL APPROACH FOR COMPLETE OR PARTIAL CALLOSOTOMY IN THE MOUSE

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Investigation of human and animal behaviour following transection of the major cerebral commissure, the corpus callosum (CC), provides valuable insights into various aspects of cerebral lateralization and intracerebral transfer of information (Bogen and Vogel, 1962; Reeves, 1991). In humans, this body of research has been contrasted with behavioural deficits following congenital abnormality or absence of the CC, a defect called agenesis of the corpus callosum (Jeeves, 1977; Jeeves, Silver, and Jacobson, 1988; Lassonde, Sauerwein, Chicoine, and Geoffroy, 1991). This genetic defect has been described only in humans and mice, permitting the potential comparison of behavioural consequences of congenital CC defects with those of CC transection only in these two species. To date, however, surgical techniques for CC transection have been developed for humans (Bogen and Vogel, 1962; Reeves, 1991), monkeys (Elliott, Ettlinger, Maccabe, and Richardson, 1976), cats (Matelli, Olivieri, Saccani, and Rizzolatti, 1983), rabbits (Russell, Van Hof, and Pereira, 1983), and, with varied success, for rats (Crowne and Richardson, 1985; Goodman and Russell, 1974; Ikeda, Sakai, and Yagi, 1992; Noonan and Axelrod, 1991), but never for mice. The technical difficulties inherent in transecting this relatively small structure in the mouse have thus far prevented attempts at developing a surgical technique for callosotomy in the mouse. In one strain of mice (CF-1), it has been found that not only can there be more than one bregma point, but due to individual differences, there are significant errors for locating structures in the frontal plane (Slotnick and Leonard, 1975). In addition, there are significant strain differences in the relative locations of lambda, bregma, and the midbrain commissures in mice (Wahlsten, Hudspeth, and Bernhardt, 1975).

In the present study, a high precision surgical approach for transection of the CC in mice was developed. This technique allows transection of parts of or the entire CC, while avoiding extracallosal damage to the colliculi, cerebellum, hippocampus, and hippocampal commissure. This approach permits later behavioural comparisons between CC transected mice and a variety of acallosal mouse strains and thus an experimental investigation of the differential effects of prenatal CC malformation with those of surgical destruction of the CC in the juvenile or adult mouse. As part of this technique, a number of improvements of apparatus and methodology which address the general issue of surgical precision are described. Limitations of the technique which result from strain and age differences are reported.

2.1. Materials and Methods

2.1.1. Animals and Anaesthesia

The subjects used were four male B6D2F2 mice, aged 44 days at the time of surgery, and weighing between 22.7 and 23.9 g. These were obtained by mating B6D2F1/J mice (the hybrid offspring of a C57BL/6J female mated with a DBA/2J male) purchased from the Jackson Laboratory (Bar Harbor, Maine).

Subjects were injected intraperitoneally (IP) with 0.1 ml of atropine sulfate (0.4 mg/ml) 30 minutes prior to anaesthesia and were then anaesthetized by IP injections of 1.3 ml/kg of pentobarbital (65 mg/ml). For local anaesthesia, 0.04 ml of Lidocaine (2.86

mg/ml) was injected under the scalp before incision. When the subjects regained consciousness, they received IP injections of 0.5 ml/kg of buprenorphine (0.04 mg/ml) for postoperative analgesia.

2.1.2. Apparatus

The knife (shown in Figure 2.1) was constructed of a 0.25 mm diameter tungsten wire of 4 cm length. One end of the wire, about 1 mm in length, was bent at a right angle and the outside edge of the hook was sharpened on 600 grain emery paper. All but this hook and the proximal 8 mm of wire were inserted into a sufficient length of stainless steel tubing (from a hypodermic needle with 0.28 mm inside diameter) to decrease the flexibility of the knife. The end of the wire, encased by the tubing, was held firmly against a stereotaxic holder in a horizontal position and aligned with the anteroposterior axis of the stereotaxic apparatus. It is important that the knife is fixed to the extreme distal end of the holder, to permit the assembly to pass over the mouse's body during surgery.

A modified Kopf professional model stereotaxic instrument with 0.01 mm graduations and a palate clamp holder (described in detail in Jones, Wahlsten, and Blom, 1977) was used. During surgery, the subject's body lay on a plastic bed heated to 37° C to prevent hypothermia. This bed could be moved relative to all three axes of the stereotaxic apparatus at any angle (Figure 2.2). Using a potentiometer connected to a voltmeter, the angle of the anteroposterior axis of the subject's body relative to the apparatus could be measured to the closest 0.1° angle.

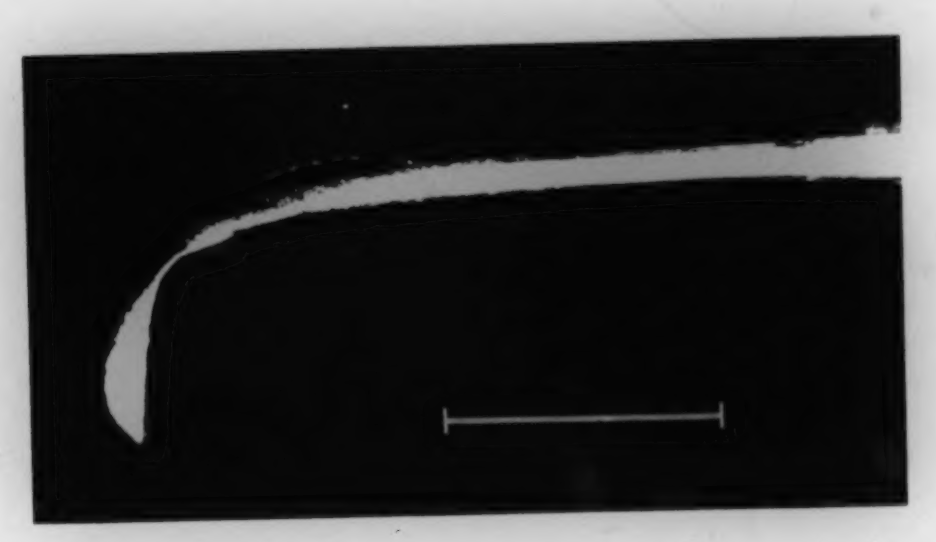

Figure 2.1. Anterior end of tungsten wire knife used during surgery. Scale bar = 1mm.

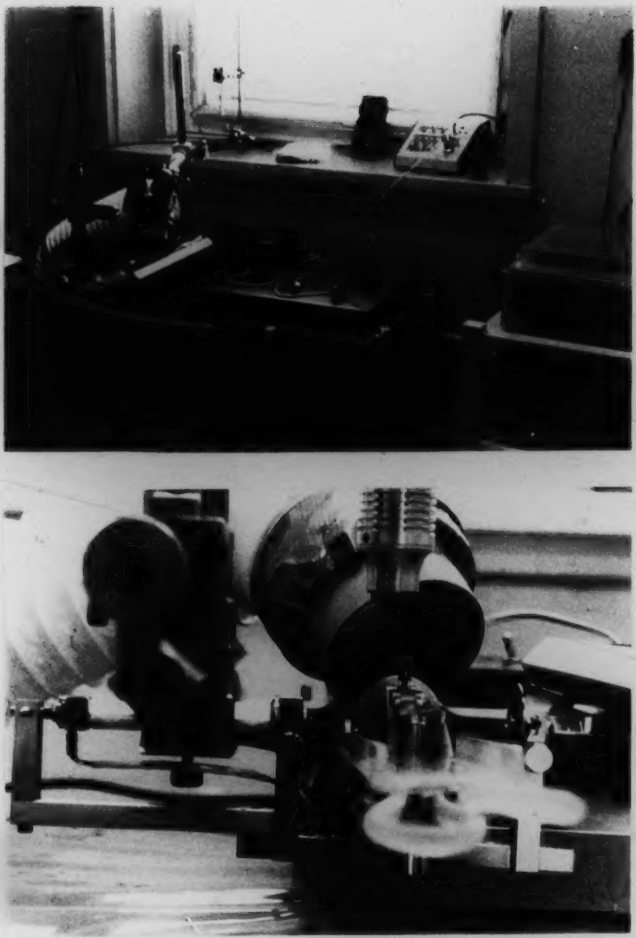

Figure 2.2. Stereotaxic apparatus used during callosotomy. Anterior to scavenge gas hose, note plexiglass bed with heating tubes, which was movable along three axes relative to the stereotaxic apparatus including the wire knife.

Because the palate clamp holder did not fix the head of the mouse in a predetermined flat skull position, the skull of subjects had to be carefully levelled after placement in the stereotaxic instrument. For this purpose a pressure sensitive, battery operated device was attached to a stereotaxic holder. This device gave auditory feedback when it was lowered onto the skull, at which point the ventral position was read on the stereotaxic instrument. Using this method, the skull was levelled to within 0.1 mm precision between lambda and bregma and between two points 2 mm lateral on either side of a point on the midline halfway between lambda and bregma.

Because the skull of mice is relatively flexible, it was important to use a high speed drill that would cut through the bone without depressing it. The drill used was a Foredom high speed lapidary drill (Series S, max. 18000 rpm) with a high speed hand piece (Foredom No. 35), which multiplies motor speed 2.5 times for a maximum drill bit speed of 45000 RPM. The drill bit used was 0.6 mm in diameter and was cylinder shaped, that is, had a flat cutting surface instead of the more common pointed or hemispherical cutting point.

2.1.3. Surgery

To minimize sources of surgical complication and distress to the animal, surgery was conducted under sterile conditions. The anaesthetized mouse was scissor shaved and placed in the stereotaxic apparatus. Subjects' eyes were covered with Polysporine sterile opthalmic ointment and the scalp disinfected with Betadine surgical scrub. The area of the incision was shaved and the scalp was cut with a #15 scalpel blade and the skull cleaned and dried with sterilized cotton tipped swabs before the skull was levelled using the head levelling device described above.

A unilateral trephine hole was drilled as a fine slot centred 0.5 mm lateral to bregma between points 1.4 mm to 3.6 mm posterior to bregma (see Figure 2.3), which generally avoided damage to the superior sagittal sinus. Bone shards were cleared from the hole and dura was broken using a pair of fine forceps. Through the single trephine hole, three separate consecutive cuts at different angles and aimed at the posterior, middle, and anterior parts of the CC were made (see Figure 2.4). For each cut, the tip of the knife was positioned on bregma with the head at the appropriate entry angle for that cut and lateral, ventral and anteroposterior positions noted for calculation of coordinates for entry. These coordinates were determined such that the knife would be positioned at 3.95 mm posterior to bregma at skull level. Because the head was not level at this time, the knife had to be moved posteriorly from bregma and then ventrally to be positioned at the entry point (see coordinates below). After the knife was inserted at an angle which would position it just dorsal to the CC, the head was moved to a steeper angle with the knife in place. In effect, this procedure resulted in lifting the CC up tightly against the underside of the knife, with the hook extending down over the genu. During withdrawal of the knife, the posterior end of the hook thus cut through the remaining CC fibres. Coordinates for the three cuts were, respectively: Cut 1 - Entry angle 43°, withdrawal angle 70°, at -2.72 mm ventral and -2.82 mm posterior to bregma, length from entry point 3.60 mm; cut 2 - entry angle 32°, withdrawal angle 46°, at -1.87 mm ventral and -2.92 mm

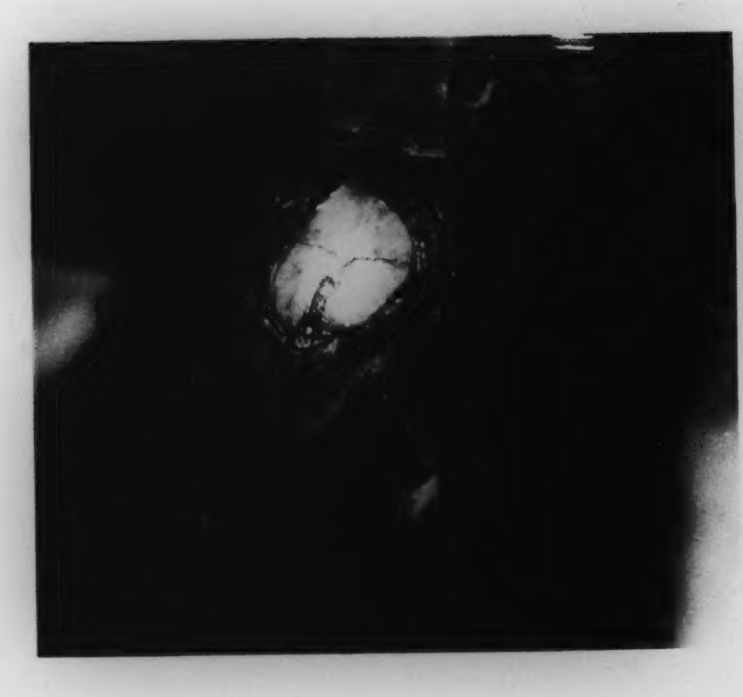

Figure 2.3. View of dorsal skull surface with unilateral trephine hole during surgery.

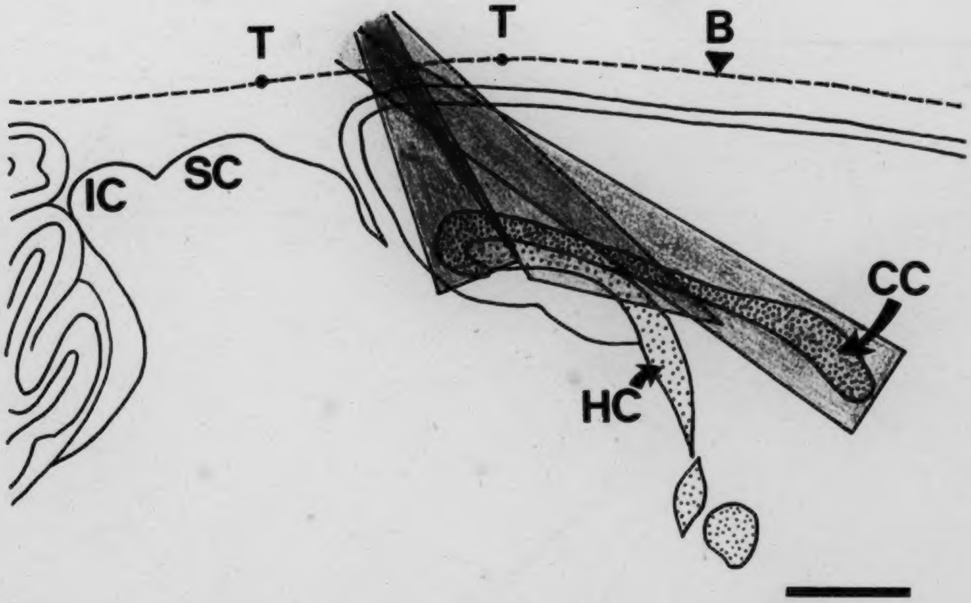

Figure 2.4. Sagittal view of mouse brain at 0.4 mm lateral to midline, illustrating effective course of knife cuts. Green area indicates course of most posterior cut, blue area indicates course of middle cut, and pink area indicates course of most anterior cut. Posterior end of the CC is shown toward left side of drawing, anterior end of the CC is shown toward right. Abbreviations: T - anterior and posterior extent of trephine hole, B - bregma, CC - corpus callosum, HC - hippocampal commissure, IC - inferior colliculus, SC - superior colliculus. Scale bar = 1 mm.

posterior to bregma, length 5.0 mm; cut 3 - entry angle 27°, withdrawal angle 37°, at -1.59 mm ventral and -3.08 mm posterior to bregma, length 6.3 mm. All cuts were made at 0.4 mm lateral to bregma and were made in the left hemisphere in two of the subjects, and in the right hemisphere in the remaining two subjects..

Any bleeding occurring during the knife cuts was stopped by application of pressure after withdrawal of the knife. After completion of all three knife cuts, the site was cleaned with sterile saline and the incision closed with sterile cyanoacrylate (Nexaband). Subjects were kept at 37° C until they regained consciousness.

2.2. Results

Two days after surgery, subjects were deeply anaesthetized with 1 ml/kg of pentobarbital (Euthanyl, 12 mg/ml IP) and perfused intracardially with isotonic saline followed by buffered 4% paraformaldehyde. Brains were removed immediately and stored in buffered 4% paraformaldehyde for four days before blocking the brains to a standard configuration. Brains were cut in coronal sections of 60 μm throughout the extent of the CC, and every fourth section was mounted on gelatin coated slides. Sections were stained with the Schmued gold chloride stain (Schmued, 1990) and permanently coverslipped. Serial coronal sections illustrating the extent of knife cuts are shown in Figure 2.5. The CC was fully transected along its entire rostrocaudal extent in all four subjects. Examination of all mounted sections from the brains of surgical subjects indicated that the extent of callosal transection was approximately 89% to 100%. This lies well within the

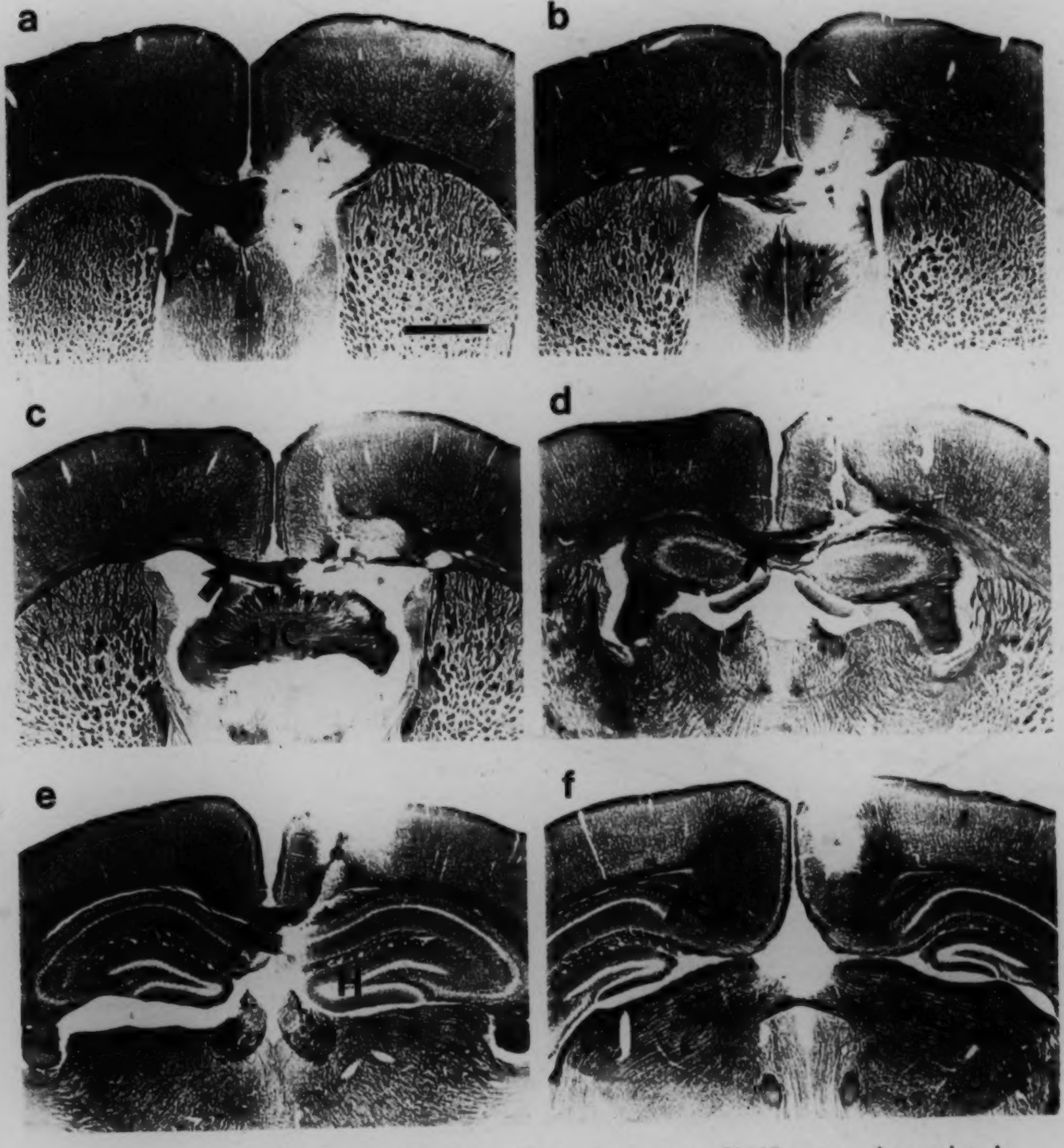

Figure 2.5 (a-f). Serial coronal sections illustrating extent of knife cuts and associated damage from most anterior extent (a), to most posterior end (f) of knife cuts. Arrow indicates corpus callosum. Abbreviations: F - fornix, HC - hippocampal commissure, H - hippocampus. Scale bar = 1 mm.

range considered to constitute a total transection in the literature on rats (Adelstein and Crowne, 1991; Denenberg, Gall, Berrebi, and Yutzey, 1986). There was no perceptible damage to the cerebellum and colliculi, and damage to the hippocampal commissure and hippocampus proper is negligible (see Figure 2.5 c-f). However, the most dorsal portion of the fornix, which is adjacent to the ventral side of the CC, is generally lesioned.

Since developing this technique, this surgery has been performed on a number of mice which were used for behavioural testing (see Chapter 4) and we have found excellent recovery from surgery and no problems with long term survival.

2.3. Discussion

This three-cut precision approach for callosotomy in the adult mouse reliably results in transection of the CC with minimal extracallosal damage. The part of the CC most consistently transected in its entire extent was the body of the corpus callosum, which carries fibres connecting the primary motor and primary and secondary somatosensory cortices (Pandya and Seltzer, 1986).

In the past, a variety of techniques has been employed to transect the CC in rats, all of which resulted in an extent of extracallosal damage which, in our opinion, would make the validity of later behavioural data questionable. In some of these techniques, an unsharpened wire knife was used to make single cuts, resulting in tearing of the surrounding tissue as well as a significant risk of cerebellar damage (Crowne and Richardson, 1985; Crowne, Novotny, and Russell, 1991; Ikeda, Sakai, and Yagi, 1992).

Another technique involved the use of a modified dental hook, which was inserted underneath the superior sagittal sinus and used to tear the CC in a non-stereotaxic procedure, resulting in significant cortical and hippocampal damage and the loss of tissue fragments (Noonan and Axelrod, 1991). A third procedure utilized a piece of surgical silk which was stretched taut between two needles inserted anterior and posterior to the CC lateral to the midline (Goodman and Russell, 1974). This method resulted in visible cortical damage and degeneration along the entire line of cutting, as well as in hippocampal damage.

Extracallosal cortical damage is unilateral and is restricted to the cingulate cortex below the trephine hole, referred to as the cytoarchitectonic field 29c (Yorke and Caviness, 1975). This area of damage receives minimal interhemispheric callosal projections (Yorke and Caviness, 1975) and lies posterior and medial to the hindlimb area and medial to the trunk area of the primary somatosensory cortex (Carvell and Simons, 1987). It receives afferent connections from the lateral and medial limbs of the lateral magnocellular nucleus of the thalamus (Vogt and Sikes, 1990) and its role in somatosensory processing is unknown (Koralek, Olavarria, and Killackey, 1990); however, it does appear to be involved in the learning of conditioned responses in rabbits (Freeman, Weible, Rossi, and Gabriel, 1997). The mouse cingulate cortex has been implicated in neural transmission between visual processing areas, though its function has not been clearly specified (Garrett, Sorensen, and Slomianka, 1992). Recent studies have found no evidence of behavioural deficits in open field tests, home cage activity, or on an eight-arm radial maze after bilateral supracallosal aspiration lesions affecting the cingulate cortex and cingulum proper (Jeltsch et al, 1994; Greene, Cassel, Kelche, Jeltsch, Bratt, and Will, 1994). It is therefore unlikely that the damage to supracallosal areas in the present study would cause significant motor deficits.

This technique can easily be modified for use in partial callosotomy. Because three separate cuts were necessary to transect the entire CC, one or two cuts could be used to section specific parts of the CC. However, the coordinates presented here are highly strain and even age-specific. As reported previously (Wahlsten, Hudspeth, and Bernhardt, 1975), there is both significant between- and within-strain variability in the structure of the skull of mice. Consequently, new coordinates must be developed when using different strains of mice or older (above 10 weeks of age) subjects with thicker skull bones. However, the principles of this precision technique could easily be adapted to achieve higher accuracy and minimal collateral damage in callosotomies not only in mice but a variety of other species.

The main drawback with the surgical technique described in the present chapter was mortality due to complications with anaesthesia, because hybrid mice display large individual differences in sensitivity to drugs. We have since overcome this drawback by using an anaesthesia machine with anaesthetic gas (isoflurane, administered at flow rates of 300 ml/min and concentrations of 2.7 to 3.5% with a Fluotec II vaporizer), which enables the surgeon to adjust the dose according to the subjects' reactions, thereby significantly reducing mortality due to anaesthesia from 5 of 19 subjects which were operated on under pentobarbital anaesthesia to 3 of 107 subjects operated on for behavioural testing under isoflurane anaesthesia. For further details on this anaesthetic technique, see Appendix 1.

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Chapter 3

CORTICAL THICKNESS IN SEVERAL STRAINS OF NORMAL, CONGENITALLY ACALLOSAL, OR SPLIT BRAIN MICE

One problem encountered when working with different strains of one species is the question of whether it is truly possible to make comparisons between strains. Behavioural differences between strains of mice, for example, can be due to the absence or presence of a specific defect of interest (such as callosal transection or agenesis), but they may also be due to a number of other incidental genetic differences between strains. These could include factors such as the degree of inbreeding, differences in body or brain size, visual acuity, or brain development, all of which may be interrelated. Some systematic genetic differences, such as fur colour *per se* (as opposed to albinism, which affects the visual system as well as fur colour), have never been shown to be linked with behaviour and thus are of little concern in behavioural research. Other genetic strain differences, such as a high degree of inbreeding as compared to a hybrid background, may be a necessary part of the experimental design. This is due to the fact that certain defects are found almost exclusively in inbred strains. In addition, hybrid animals are often preferred as control subjects with a highly variable genetic background and therefore with a low probability of being homozygous recessive for characteristics which reduce evolutionary fitness in the Darwinian sense.

Other systematic genetic differences between strains which could affect many behaviours can be controlled statistically for strain differences. In the series of behavioural tests described in Chapter 4 of the present thesis, for example, data were statistically corrected for strain differences in overall brain size, by using relative brain size of subjects compared to other mice from the same genetic background. However, there is one other major factor, brain development, specifically the extent of neocortical growth, which has a significant influence on many complex behaviours. Norton (1989), for example, has shown that cortical thickness in rats can in fact be predicted by a number of behavioural measures such as the pattern of leg movements associated with gait. Thus far no data about possible strain differences in cortical thickness has been available for the strains used in the present program of research; therefore, it is unclear whether there should be a statistical correction of data for this factor as well.

Numerous factors can affect neocortical development or thickness of the neocortex. Juraska and Reid (1994), for example, found nonsystematic changes in the cortical thickness of rats as a result of the transient hormonal changes associated with pregnancy. Cortical thickness in mice is also decreased as part of a pattern of overall brain deficits resulting from chronic exposure to ethanol during fetal development (Wainwright, Levesque, Krempulec, Bulman-Fleming, and McCutcheon, 1993). Of particular interest in the present program of research is the fact that at least two studies have shown evidence of a link between callosal development and neocortical abnormalities. In human fetuses, polymicrogyria of the cortex is associated with callosal agenesis and may be due to the fact that the failure of the callosum to develop occurs at the stage of neural development when neuron proliferation and neuronal migration to the cortex take place (Stefanko and Schenk, 1977). Another study found that three strains of inbred mice (NZB, DBA, and BXSB) with significant differences in callosal areas exhibited concurrent differences in neocortical volume, with a significant, positive correlation between midsaggital callosal area and total neocortical volume (Rosen, Sherman, Emsbo, Mehler, and Galaburda, 1990).

Considering the latter findings, it is possible that neocortical thickness could also differ significantly in a number of other mouse strains with varying degrees of abnormality of the corpus callosum, some of which were used for behavioural testing described in Chapter 4 of this thesis. If these strains differed significantly in cortical thickness, any behavioural differences that exist between them could be due to differences in the degree of cortical development rather than callosal pathology. This would make a meaningful interpretation of behavioural differences impossible. The present study therefore compared neocortical thickness at various positions in the brains of eight different mouse strains with varying degrees of congenital or acquired callosal pathology.

3.1. Materials and Methods

3.1.1. Subjects

A total of 70 mice of both sexes aged between 55 and 369 days from strains with different degrees of callosal defects were used. By the age of 45 days the mouse corpus callosum is almost completely matured (Sturrock, 1980); therefore, the degree of callosal abnormality is fully apparent in mice of that age range. Between seven and ten mice from each of eight different genetic backgrounds were used. Two of these strains, namely B6D2F$_1$ (the F$_1$ hybrid cross between the inbred strains C57BL/6J and DBA/2J) and RI line 11 always have normal callosa. Five strains used, namely ddN, I/LnJ, BALB/cWah1, 129/ReJ, and RI line 19 have different degrees of penetrance of congenital absence of the corpus callosum with individual callosal sizes ranging from normal to complete absence.

The recombinant inbred (RI) lines were derived from the F_2 cross of 129/ReJ and BALB/cWahl inbred by full-sib mating for between four and eight generations at the time of this study (see Wahlsten and Schalomon, 1994). One group of subjects was composed of B6D2F_2 mice (the F_2 hybrid cross between the inbred strains C57BL/6J and DBA/2J) which had undergone surgical transection of the corpus callosum along its entire length (as described in Schalomon and Wahlsten, 1994) approximately two months before sacrifice. No B6D2F_2 control subjects were used, as data on the B6D2F_1 subjects were previously available, and ancestry is shared between the F_1 and F_2 hybrids. Some subjects had been used for breeding or behavioural testing before perfusion and histological processing. Those subjects which had been used for behavioural testing had been tested on a running wheel or a balance beam (as described in Chapter 4) for a maximum of five days of testing .

3.1.2. Histology

Mice were injected with an overdose of sodium pentobarbital (120 mg/kg IP), and perfused intracardially with approximately 10 ml of normal saline followed by 20 ml of neutral 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were immediately extracted from the skull and placed in fresh fixative for a minimum of seven days. After this time, they were blocked to a standard configuration (see Wahlsten, 1984), blotted dry, and weighed. Coronal sections were cut on a freezing microtome at 30 microns thickness throughout the entire extent of the forebrain. Every fifth section was mounted on gelatin coated slides, stained with gold chloride (Schmued, 1990), and permanently coverslipped.

For each brain, four tracings of sections were made at 25 times magnification using a drawing tube. The first section chosen for tracing was the section immediately posterior to the first section in which the forceps minor of the CC was visible in both hemispheres as a dense, continuous mass of stained fibres. The second section traced was the most anterior section in which anterior commissure fibres were crossing the midplane. The third section traced was the most anterior section in which the dentate gyrus of the Hippocampus was visible as an arrowhead shaped structure in both hemispheres of the brain. The last section traced was the most anterior section in which posterior commissure fibres were crossing the midplane. Tracings showed the outline of the cerebral cortex along the midline and laterally to where the dense plexus of cortical axons extending from the callosum is no longer visible. In addition, the upper limit of the callosum was traced. In the rare cases where a parts of a section had detached from the slide during staining and shrunken, the probable true surface of cortex was drawn with a dotted line and used in cortical thickness measurements. Where cracks were detected in the cortex, the crack was traced and not included in thickness measurements.

In addition, two tracings of the CC at the midplane and of adjacent structures were made at 160 times magnification at the most anterior levels of midline crossing by the anterior and hippocampal commissures. The thickness of the CC at these two points was averaged for the purpose of statistical analyses.

Cortical thickness was defined as the thickness of the cortex from the surface of cortical layer I to the medial border of layer VI. This method has been employed in past research (Juraska and Reid, 1990), although some have measured the thickness of each cortical layer separately (Norton, 1989), or have included only cortical layers II to IV (Miki, Fukui, Uemura, and Takeuchi, 1994). In the present study, cortical thickness was measured across all six cortical layers because this ensured that changes in thickness at any layer of the cortex would be detectable in the data. In addition, the myelin stain used for histology did not permit visualization of separate cortical layers. Thickness was measured at five points in each section (see Figure 3.1). Lateral thickness was defined as the distance of a line perpendicular to the lateral cortical surface to the point on the lateral edge of the CC where dense fibres projecting from the CC to the cortex first become apparent under the light microscope. This point was defined as the ventral edge of the CC, and it is readily apparent in myelin-stained coronal sections (see Figure 3.2). Cingular thickness was defined as the length of a perpendicular line between the cingular cortex and the point where the CC becomes deflected sharply ventrally before crossing the midplane. Medial thickness was defined as the length of a line from the same point on the CC, and perpendicular to the interhemispheric fissure. In addition, cortical thickness was measured at two additional points which were defined not by anatomical structures, but which were spaced at even distances between the points at which the lateral and the medial cortical thicknesses had been measured. Dorsal thickness was measured one third of the way from the cingular point on the cortex and CC to the lateral points and midway thickness was measured two thirds of the way between the same points.

All measurements of cortical thicknesses were done separately for the right and the left hemisphere and averaged between hemispheres. An exception to this method was made in brains from subjects of RI lines 11 and 19. These had been sagitally bisected in

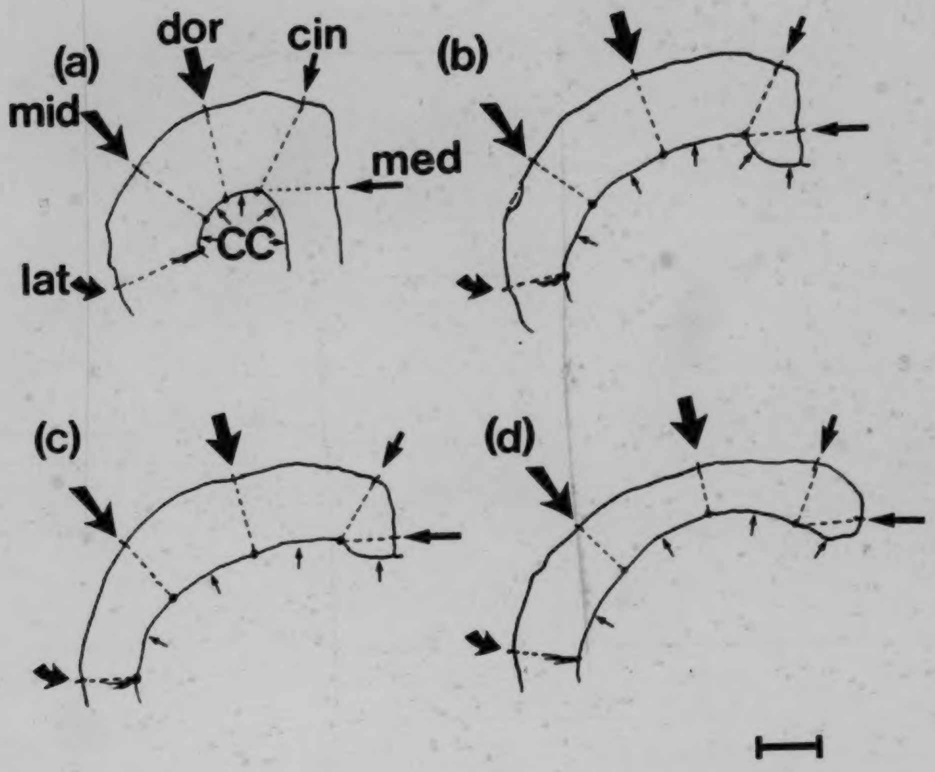

Figure 3.1 (a-d). Sample tracings of coronal sections of a mouse brain at the level of (a) anterior end of CC, (b) the anterior commissure, (c) dentate gyrus of the hippocampus, and (d) the posterior commissure. In each tracing, the cortical thickness at lateral (lat), midway (mid), dorsal (dor), cingular (cin), and medial (med) points are indicated. Small arrows indicate the dorsal edge of the CC. Scale bar = 1 mm.

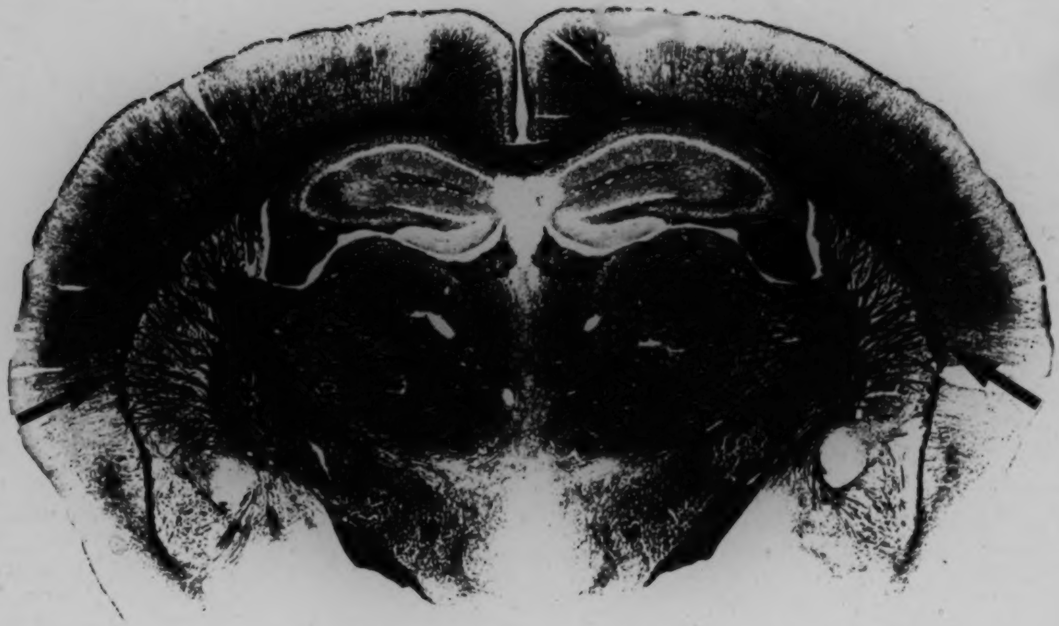

Figure 3.2. Coronal section of mouse brain stained with gold chloride showing the dense fibers projecting from the CC to the cortex (see arrows) which were used to define the most ventral extent of the CC and therefore lateral cortical thickness.

prior research, and only the right hemisphere was available for tracing. Another exception was made in the B6D2F$_2$ subjects which had undergone surgery. In that group, measurements were done only in the hemisphere contralateral to surgery (which was the right hemisphere in half of the brains), and raw measurements rather than averages were used in all analyses.

3.1.3 Statistical Analyses

As part of the statistical analyses of the data performed both for the cortical thickness data in the present chapter and for behavioural data presented in Chapter 4, Pearson *r* correlation tables of large numbers of correlations were calculated. If there are many correlations, unadjusted *p* values are not the true probabilities, because they do not reflect the number of correlations being tested. Therefore, statistical correction procedures, such as a Bonferroni correction, have to be applied. The Bonferroni correction can be applied directly to α, the probability of a Type I error, by computing $\alpha/\sqrt{N}$. This means that the *p* value needed to achieve statistical significance will vary with the number of correlations that are computed for any set. Alternatively, it is acceptable to apply the Bonferroni correction directly to the achieved significance level *p*, such that the corrected *p* values listed in correlation tables do reflect the number of tests that were performed. This makes interpretation of significance easier, because regardless of the number of correlations in any set, the same adjusted *p* value can be used to determine statistical significance. This is the procedure chosen for all correlations computed for the current and the following chapter. Corrections were applied by the Systat statistical program and were computed as corrected $p = p$ * number of correlations, such that the total α value for the combined number of correlations did not exceed the level chosen for significance (Wilkinson, 1990, pp. 63-64 and 239).

3.2. Results

On inspection, it became apparent that the most anterior sections drawn from each brain would not be useful for analysis. The most anterior edge of the CC, where the tracing was obtained, is oriented dorsoventrally such that the coronal crossectional area of the callosum increases drastically between adjacent coronal sections, and cortical thickness decreases accordingly. Because the sections which had been retained for tracing were spaced at 150 μm distance from each other, the actual thickness of the cortex dorsal to the CC in the tracings depended mainly on the placement of the section relative to the anterior edge of the CC, rather than on cortical characteristics of the brain in general. The most anterior measurement of cortical thickness from each brain was therefore discarded from analysis.

In order to reduce the number of independent variables used in analyses and thereby increase the power of statistical tests, analyses were performed on the average cortical thickness at the five points of measurement (lateral, midway, dorsal, cingular, and medial) across the three tracings retained for analysis. Due to problems with histology, some data were missing for one subject of the BALB/cWah1 strain, and this subject was dropped from the data set. Thus, 69 mice remained in the final data set.

To verify whether subjects of each of the eight groups were comparable with respect to sex, age, brain weight, body weight, and CC thickness at the midplane, *F* tests were performed (see Table 3.1). There were significant differences between groups with respect to age, body weight, brain weight, and CC thickness. RI mice of both lines 11 and 19, and to some degree B6D2F$_2$ mice which had undergone surgery, were younger than mice of all other groups (F=11.4, p<.001), and had the lowest body weights (F=10.4, p<.001). This was due to the fact that these subjects had been used in behavioral research and had been sacrificed after behavioral testing. Strain typical differences in brain weight (personal observation, see also Wahlsten and Andison, 1991) were also found, with BALB/cWah1 and 129/ReJ subjects having the heaviest brains, and ddN and I/LnJ mice having the smallest brains (F=16.2, p<.001). Two of the eight strains were completely acallosal, having CC thicknesses at the midline of 0 mm (I/LnJ and RI line 19), subjects in four of the strains had average CC thicknesses above 0.2 mm (ddN, RI line 11, B6D2F$_1$, and 129/ReJ), and two strains had CC thicknesses in the intermediate range (BALB/cWah1 and surgery).

F tests were also performed to verify whether there were any a priori group differences in cortical thickness (see Table 3.2). All strains tended to show similar overall cortical topography, with cortex being thick at the midway point of measurement and thin at the medial point. There were significant group differences on all cortical thickness measures (F=8.84 to 30.3, p<.001). Cortical thickness across the five points of measurement tended to be largest in I/LnJ mice, RI line 19, RI line 11 and surgery mice

Table 3.1

Descriptive Statistics (Means and Standard Deviations) and χ^2 and F tests for Group Differences on Independent Measures and Average CC Thickness at the Midplane

		Sex*		Age		Brain Weight		Body Weight		CC Thickness	
	n	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Normal CC:											
RI line 11	10	0.500	.527	69.3	9.8	0.465	.011	22.3	2.30	0.266	.052
B6D2F$_1$	7	0.429	.535	177.4	47.4	0.435	.020	34.8	6.81	0.264	.062
Incomplete Penetrance of ACC:											
ddN	8	0.500	.535	222.5	78.4	0.429	.026	32.6	3.42	0.242	.111
BALB/cWah1	7	0.429	.535	234.9	129.1	0.449	.032	28.2	4.30	0.047	.071
129/ReJ	10	0.400	.516	191.4	76.4	0.480	.023	32.6	3.14	0.207	.180
Total ACC:											
I/LnJ	7	0.571	.535	211.4	8.9	0.429	.013	26.1	2.81	0.000	.000
RI line 19	10	0.500	.527	60.8	3.0	0.499	.009	23.8	4.30	0.000	.000
Callosotomized:											
B6D2F$_2$	10	0.300	.483	130.0	46.5	0.439	.012	25.8	3.61	0.093	.063
Test:		χ^2=1.74 (7df)		F=11.1		F= 16.2		F=10.4		not defined	
p		.973		<.001		<.001		<.001			

* coded +1 for males, -1 for females

Table 3.2

Descriptive Statistics (Means and Standard Deviations) and F tests for Group Differences for Cortical Thickness Measures (in mm)

		Lateral		Midway		Dorsal		Medial		Cingular		Overall Mean	
	n	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Normal CC:													
RI line 11	10	1.02	.064	1.17	.044	1.14	.056	0.77	.043	1.06	.046	1.03	.037
B6D2F$_1$	7	0.92	.035	1.00	.031	0.92	.046	0.76	.017	0.98	.039	0.92	.019
Incomplete Penetrance of ACC:													
ddN	8	0.96	.034	0.98	.032	0.89	.026	0.78	.048	0.89	.084	0.90	.035
BALB/cWahl	7	0.95	.049	1.02	.035	0.97	.050	0.87	.063	1.00	.068	0.96	.040
129/ReJ	10	0.98	.057	1.03	.053	0.96	.042	0.86	.064	0.96	.049	0.96	.043
Total ACC:													
I/LnJ	7	1.00	.050	1.08	.041	1.01	.040	0.97	.046	1.14	.037	1.04	.033
RI line 19	10	1.08	.065	1.18	.048	1.09	.046	0.92	.045	1.06	.052	1.07	.027
Callosotomized:													
B6D2F$_2$	10	0.92	.065	1.08	.044	1.01	.056	0.87	.071	1.02	.077	0.98	.050
F		8.8		27.1		30.3		15.5		12.9		21.3	
p		<.001		<.001		<.001		<.001		<.001		<.001	

and smallest in ddN and B6D2F$_1$ mice. Thus, mice with varying degrees of callosal development were among those groups with greatest and those with smallest cortical thickness.

In order to analyse the relationship between these independent measures, CC thickness at the midplane and cortical thickness measurements, Pearson correlations were calculated (Table 3.3). Several thickness measures were significantly correlated with age, brain weight, and body weight, indicating that the main determining factor for cortical thickness was the general size of the subject. This general size factor appeared to affect all areas of the cortex; cortical thicknesses were also significantly correlated with each other. Thickness of the CC at the midplane was significantly correlated with only two of the cortical thickness measures, medial thickness ($r=-.777$, $p<.001$) and cingular thickness ($r=-.431$, $p=.010$). This indicates that CC thickness at the midplane was related only to the thickness of cortex directly at the midplane, adjacent to the callosum at the point of decussating, such that in brains with thinner callosa at the midplane, the cingular and medial cortices were thicker. At more lateral points, where callosal axons are found even in completely acallosal brains, cortical thickness was not significantly correlated with callosal malformation. Scatter plots of mean overall cortical thickness against CC thickness for all subjects pooled and for each of the strains of subjects separately also fail to show any relationship of overall cortical development with CC defects (Figure 3.3).

Multiple regression analyses were performed to test whether any of the cortical thickness measures could be reliably predicted by group, sex, age, brain weight, body weight, or average CC thickness at the midplane (see Table 3.4). Effect coding was used

Table 3.3

Correlations (Pearson *r*, with Bonferroni Adjusted Probabilities),
Between Independent Variables, Cortical Thickness Measures, and CC Thickness at the Midplane

	Brain	Body	Lateral	Midway	Dorsal	Medial	Cingular	CC Thickness
Age	-.280	.546**	-.364	-.623**	-.634**	.055	-.262	.056
Brain Weight		-.127	.484**	.522**	.401	.179	.090	-.008
Body Weight			-.359	-.598**	-.656**	-.150	-.473*	.266
Lateral				.718**	.588**	.303	.428*	-.182
Midway					.904**	.244	.612**	-.225
Dorsal						.121	.650**	-.137
Medial							.521**	-.777**
Cingular								-.431*

Notes: * p <= .01
** p <= .001
[a] groups coded 0-7 for eight mouse strains
Source of Bonferroni adjustment of p is (p*number of correlations)

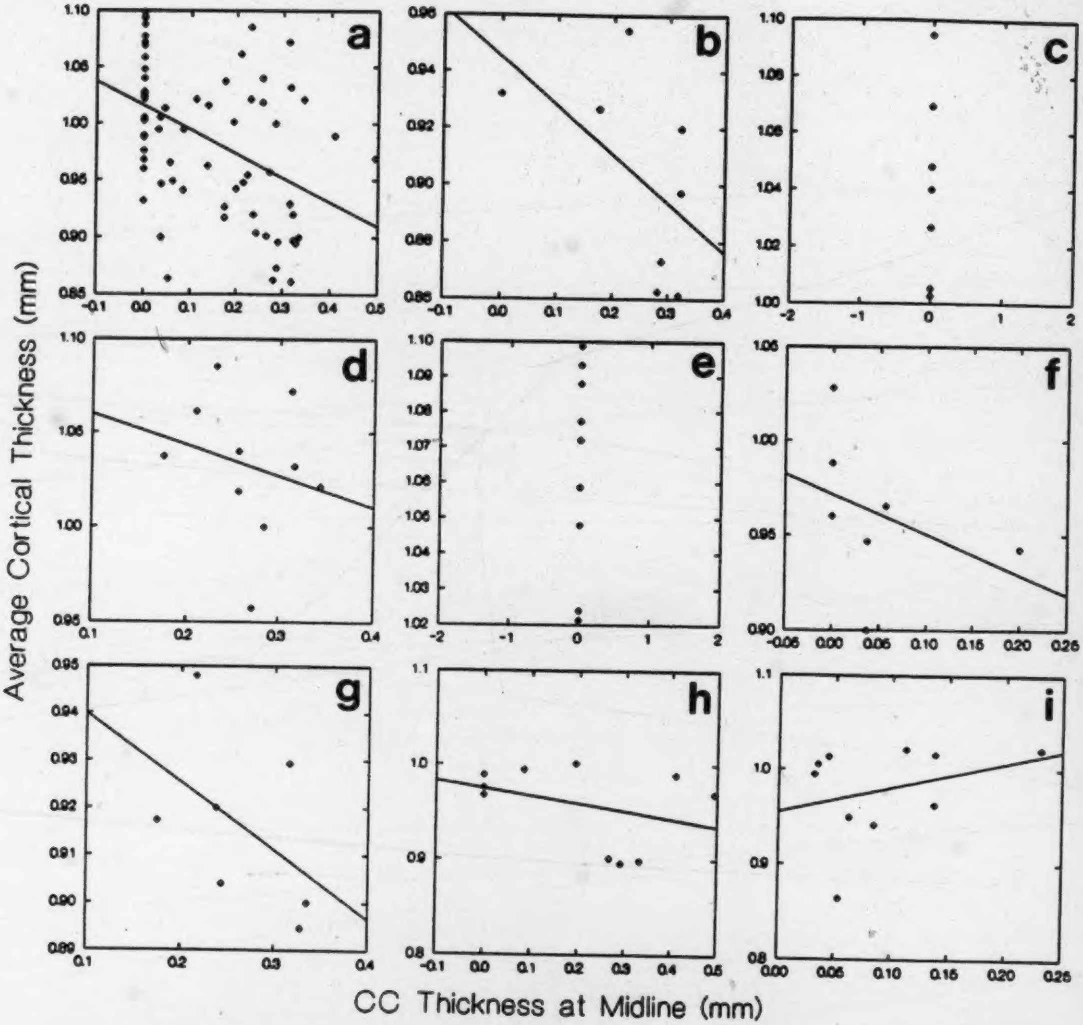

Figure 3.3 (a-i). Scatter plot of mean cortical thickness (averaged over three anterio-posterior levels and five points of measurement) for (a) all subjects pooled, (b) ddN, (c) I/LnJ, (d) RI 11, (e) RI 19, (f) Wahl, (g) B6D2F$_1$, (h) 129Re/J, and (i) callosotomized B6D2F$_2$ subjects. Regression line is shown. There is no regression line for plots (c) and (e) due to a lack of variance on one variable.

for all nominal independent variables and regression was performed stepwise backwards. As a result, predictors were excluded from the model until all remaining predictors met the criteria for inclusion in the model. Minimum significance for inclusion of predictors was set at *p*=.015, and the minimum tolerance for predictions was .98, such that only predictors that were highly independent of each other could be used simultaneously as predictors for the dependent measure. Results of the regression analyses closely parallelled results from earlier analyses. Cortical thickness close to the midplane could be predicted by callosal thickness at the midplane, whereas cortical thickness more laterally was reliably predicted by independent measures related to overall size. Group was a predictor only for lateral cortical thickness, which was greatest in two acallosal strains (I/LnJ and RI line 19) and one strain with normal CC (RI line 11), and smallest in ddN mice, which have normal callosa (see Table 3.2).

3.3. Discussion

Cortical thickness was measured in eight strains of mice with intact CC, varying degrees of congenital absence of the CC or transection of the CC, in order to determine whether callosal morphology is related to general cortical growth. If congenital malformation or surgical ablation of the CC did induce a general decrease in cortical development, behavioural deficits due to absence of the CC might be due to these cortical changes rather than the absence of interhemispheric callosal connections.

One possible source of error in the present study arises from the fact that cortical

Table 3.4
Multiple Regression Analysis of Cortical Thickness Measures

Cortical Measure	Predictor(s)	Standardized Coefficient	p (2-tailed)	Adjusted R^2
Cingular	Body Weight	-.385	<.001	.303
	CC Thickness	-.328	.003	
Medial	CC Thickness	-.777	<.001	.598
Dorsal	Age	-.317	.002	.580
	Brain Weight	.255	.003	
	Body Weight	-.450	<.001	
Midway	Age	-.307	.002	.604
	Brain Weight	.388	<.001	
	Body Weight	-.381	<.001	
Lateral	Age	-.254	.010	.424
	Brain Weight	.462	<.001	
	Group*	-.401	<.001	

* coded 0-7 for eight strains of subjects

thickness in surgery subjects was measured only in the hemisphere contralateral to surgery. Using both hemispheres of surgical subjects for cortical thickness measures might have introduced effects of several extraneous variables into the data set, thus making analysis of the data less meaningful. The main problem with the hemisphere ipsilateral to surgery was that some cortical damage in the cingular cortex in posterior sections always existed as a direct result of surgery, thus making thickness measurements at certain points impossible. In addition, cortical thickness in that hemisphere could have been affected by swelling after surgery or by gliosis and degeneration around the site of the cortical damage. Cortical thickness in the hemisphere ipsilateral to surgery was therefore never measured, however, it did not appear grossly different from that of the contralateral hemisphere upon visual inspection.

While there were significant group differences in cortical thickness at all five points of measurement, these differences did not appear to be systematic. Even when cortical thickness was adjusted for age differences, no single group consistently had the greatest or smallest cortical thickness overall, and strains of mice with both normal and acallosal brains were found among those who tended to have a thicker or thinner cortex than average. The fact that there were no systematic relationships between strain and cortical thickness was also indicated by the absence of correlations between these measures. The measure that appeared to be most strongly and consistently correlated with cortical thickness was the general size of the subject. This lack of a significant correlation between group membership and cortical thickness is in conflict with the significant group differences in cortical thickness found in descriptive statistics. The conflict may be due to the highly conservative Bonferroni correction applied to significance levels in the correlation matrix. The most likely explanation for the conflict can be found in the fact that the coding system for the eight groups of subjects used in statistical analyses was purely nominal, not ordinal, and thus did not reflect group differences in callosal pathology.

Only the thickness of medial and cingular cortex was significantly, albeit negatively, correlated with CC thickness at the midplane, the best indicator of CC morphology in the present data. In brains with congenital absence of the CC, callosal axons are deflected from the midline, and form a large Probst bundle which is located somewhat ventrolateral from the normal point of decussation of callosal axons (Stefanko and Schenk, 1977). Because the relatively large bundle of fibres that constitutes the normal callosum is missing near the midplane in acallosal brains, the space usually occupied by the CC may be dedicated to cerebral cortex, resulting in greater cortical thickness near the midplane in these brains (Figure 3.4). This conclusion was confirmed by results from multiple regression analyses, which indicated that callosal size in the present set of brains could reliably predict cortical thickness only at medial and cingular points of measurement.

While thickness of the CC at the midplane was the best available indicator of callosal morphology in the present set of data, it must be remembered that the value of this measure was mainly in determining whether a given brain was acallosal (CC thickness of the midplane, a thicker CC does not generally indicate a larger overall CC area. Rather, 0), or whether a CC was present. Wahlsten (1987) has shown that in brains with a CC at

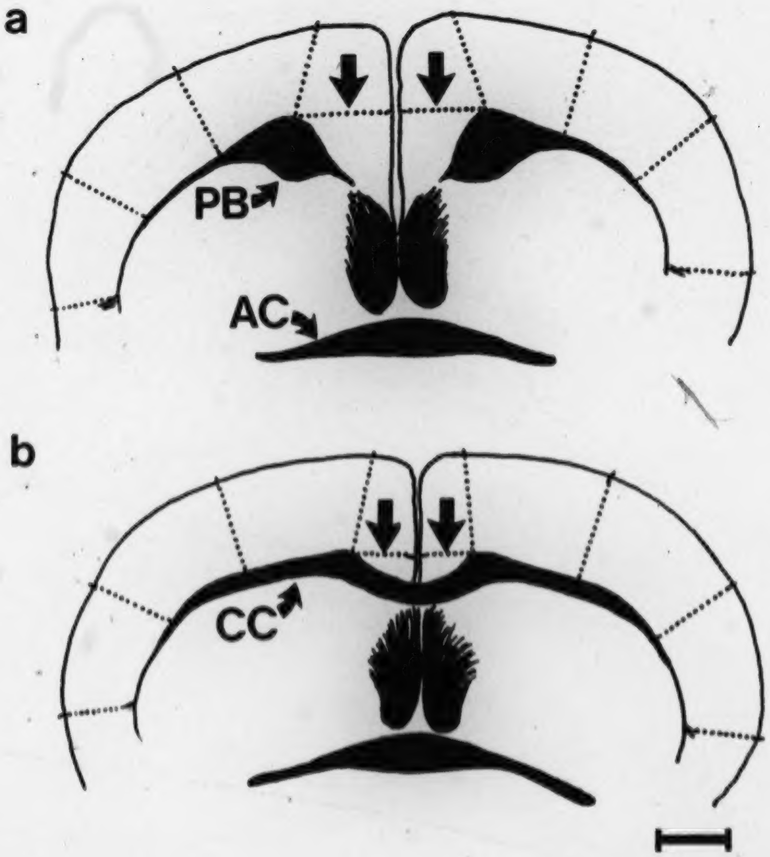

Figure 3.4. Sample tracing of (a) acallosal brain showing Probst Bundle and (b) normal brain showing CC. Note the decrease in thickness of medial cortex (indicated by dotted line) in the acallosal brain. Abbreviations: CC - corpus callosum, PB - Probst Bundle. Scale bar = 1 mm.

CC thickness at the midplane is actually somewhat greater in a short, partially absent CC, which tends to be thicker at the midplane. However, in the present data set, the variance in CC thickness could be accounted for mainly by whether a CC was present or not. In addition, one of the two strains with CC thickness intermediate between completely acallosal and normal brains was the group of surgery mice in whom lower CC thickness at the midplane truly does indicate fewer non-transected CC fibres rather than a large, thin CC. It is therefore unlikely that there was a true correlation between cortical thickness measures and CC morphology.

The data therefore strongly indicate that cortical thickness in acallosal brains is not consistently reduced relative to that in normal brains. The main determinant of cortical thickness is overall growth, which is also evident in body and brain size of the animal. Near the midplane, where there is a significant relationship between cortical thickness and callosal morphology, cortical thickness is in fact increased in acallosal strains. Therefore, if behavioural deficits were found in acallosal mice, these deficits should not be due to cortical dysgenesis. Mild cortical overdevelopment, as observed in acallosal mice in this study, if anything should confer a relative advantage on behavioural tests to these mice. IQ in humans, for example, is positively correlated with gray matter volume (Andreasen, Flaum, Swayze, O'Leary, Alliger, Cohen, Ehrhardt, and Yuh, 1993). One study using rats confirmed that cortical thickness is related to behaviour in rodents as well. Norton (1989) was able to predict cortical thickness in rats exposed to varying amounts of ionizing radiation *in utero* by their behavioural profiles; unfortunately the author failed to indicate the direction of behavioural change in rats with abnormally thin cortex.

Obviously, the present study cannot presume to indicate that extracallosal brain development in mice with various degrees of congenital or acquired CC abnormality is completely normal. Only cortical thickness was assessed in the present experiment, but it appears to be largely unaffected by CC morphology. Future research should focus on whether the subcortical motor systems, foremost the basal ganglia, are affected by CC abnormality. Certainly the Probst bundle is localized such that it may interfere with normal development of the basal ganglia. If this were the case, motor behaviours could be compromised by congenital absence of the CC.

Surgical ablation of the CC, when done during adulthood, as in the present sample of split brain mice, could not affect early brain development. It would be interesting to see whether split brain surgery in the early postnatal period would affect brain development; however, at present no good surgical method for split brain surgery in infant mice is available. On the other hand, surgical ablation in adulthood could lead to cortical changes due to degeneration of CC axons or even due to transneuronal degeneration. To control for this effect, brains of surgical subjects sacrificed at varying lengths of time after surgery could be studied. During the early postsurgery period, no degeneration could have occurred yet; however, swelling of the proximal axon stump might occur. At longer intervals after surgery, degeneration could lead to thinning of the cortex. Due to the fact that most transneuronal degeneration resulting from callosal transection would occur during the first one or two months postsurgery, it is unlikely that longer postsurgical periods than used in the present study would result in significant increases in postsurgical morphological changes.

At present, we can therefore merely assume that possible behavioural differences in mice with congenital or acquired CC deficits may indeed be due to a lack of intrahemispheric integration and are not results of generalized cortical atrophy.

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Chapter 4

BEHAVIOURAL TESTS OF MOTOR COORDINATION IN CONGENITALLY ACALLOSAL AND SPLIT BRAIN MICE

The corpus callosum (CC) is the largest fibre tract involved in the transfer of information between homo- and heterotopic areas of the cerebral hemispheres (Lomber, Payne, and Rosenquist, 1992). When the CC is surgically transected in adults, usually as a treatment for severe epilepsy, a classic array of deficits called the split brain syndrome is observed (Sperry, Gazzaniga, and Bogen, 1969). While consequences vary with the extent of transection (Jeeves, 1991) and with age at surgery and recovery time (Jinkins, 1991; Lassonde, Sauerwein, Geoffroy, and Decarie, 1986), patients are severely impaired at tasks requiring the interhemispheric transfer of information. The best documented deficit occurs in tasks requiring right hemisphere access to language (Sperry, Gazzaniga, and Bogen, 1969), but tactile discrimination (Lassonde, Sauerwein, Geoffroy, and Decarie, 1986), binocular fixation (Jeeves, 1991), and transfer of visual information (Risse, Gates, Lund, Maxwell, and Rubens, 1989) are also impaired. Motor deficits after CC transection are often transient (Provinciali, DelPesce, Censori, Quattrini, Paggi, Ortenzi, Mancini, Papo, and Rychlicki, 1990), yet disruption of transfer of complex learned motor sequences has been observed in both monkeys (Eacott and Gaffan, 1990) and humans (Bogen and Bogen, 1988) with chronic split brain syndrome.

By contrast, subjects with congenital absence of the CC (ACC) do not exhibit this syndrome and may in fact be almost asymptomatic (Bruyer, Dupuis, Ophoven, Rectem and Reynaert, 1985), although a large proportion of human subjects with ACC have associated neurological anomalies (Andermann, 1981; Wisniewski and Jeret, 1994). In those with pure ACC, the most common symptomatology is mental retardation (Lacey, 1985; Serur, Jeret, and Wisniewski, 1988), likely due to deficits in memory consolidation resulting from the CC defect (Geffen, Forrester, Jones, and Simpson, 1994). Patients with ACC do appear to have access to language function with either hand (Lassonde, Sauerwein, Chicoine, and Geoffroy, 1991) and only subtle tactile deficits (Lassonde, Sauerwein, McCabe, Laurencelle, and Geoffroy, 1988). Visual transfer of information has variously been described as completely absent (Ettlinger, Blakemore, Milner, and Wilson, 1972) or almost normal (Lassonde, Sauerwein, McCabe, Laurencelle, and Geoffroy, 1988), depending on task demands. On the other hand, there are many reports of motor deficits in ACC, from a general inferiority on sensorimotor learning tasks (Sauerwein, Nolin, and Lassonde, 1994) to performance scores on IQ tests which are notably lower than verbal scores (Bigler, Rosenstein, Roman, and Nussbaum, 1988). Performance on fine motor tasks, such as stringing beads or wrapping a string around a pencil (Reynolds and Jeeves, 1977; Field, Ashton, and White, 1978) is very slow. In general, ACC patients appear to have the greatest deficits on tasks requiring very precise, complex, or fast responses.

The classic task employed to test bimanual motor coordination in humans involves an apparatus not unlike an etch-a-sketch children's toy. Two crank handles have to be turned to draw diagonal lines with a plotter, such that one hand controls vertical displacement of the pencil and the other horizontal displacement (Preilowski, 1977). Both subjects with surgical transection of the CC and patients with ACC have been tested on this task (Jeeves, Silver, and Jacobson, 1988) and were impaired relative to normal controls. Patients with transections of the entire CC or the anterior CC showed no improvement with practice and required twice as much time as controls. ACC patients also were unable to match controls with respect to speed or accuracy and relied heavily on visual feedback (Jeeves, Silver, and Milne, 1988).

It is evident that both split-brain patients and acallosals have deficits on tasks involving interhemispheric transfer of information. The extent and quality of these deficits are not equivalent between these groups, however. Deficits in split-brain patients are dependent on the extent of surgery but are generally more severe than in acallosals. There is some evidence for greater deficits in acallosal patients on bimanual coordination tasks, particularly when task demands are high. The most cogent explanation for these facts is that in adult surgical patients the only possible compensatory mechanisms for the neurological defect are behavioural cross-cueing strategies, whereas in the congenital condition there is sufficient neural and functional plasticity to provide some degree of interhemispheric transfer.

Four main theories of compensation in ACC have been proposed. Some functions in the acallosal brain may be bilaterally represented, which would reduce the need for interhemispheric communication (Ettlinger, Blakemore, Milner, and Wilson, 1972). However, bilateral representation cannot account for recent evidence on abilities of acallosals to compare stimuli delivered simultaneously to both hemispheres (Lassonde, Ptito, and Lepore, 1990). The possibility of cross-cueing is not very plausible either, because reaction times in ACC patients are too short to be accounted for by cross-cueing strategies and little need for such strategies would exist outside laboratory settings.

A third possible compensatory mechanism concerns alternate routes of interhemispheric transfer. The most frequent suggestion is that existing non callosal commissural fibres, such as those of the anterior commissure (AC), may be increasingly used or developed, although evidence supports this hypothesis only moderately. A rerouting of CC fibres through the AC in mice has not been found (Wahlsten and Jones, 1983; Ozaki, Murakami, Toyoshima, and Shimada, 1987), nor do the size of the AC and the number of myelinated AC axons differ between acallosal and normal mice (Livy, Schalomon, Roy, Zacharias, Pimenta, Lent, and Wahlsten, 1997). There is a moderate (21%) increase in the number of unmyelinated AC axons in acallosals, although the total number of additional fibres in these mice is far less than the number of missing CC fibres (Livy et al, 1997). There is as yet insufficient evidence to prove whether other crossed fibres, such as the intertectal commissure (Milner, 1994) or direct thalamo-cortical fibres (Carretta, Sbriccoli, Santarelli, Pinto, Granato, and Minciacchi, 1996) may also be involved in compensating for the CC defect. However, recent evidence (Morin, Dolina, Robertson, and Ribak, 1994) indicates that at least one acallosal BALB/c mouse substrain displays aberrant projections to the diagonal band of Broca and the medial septal nucleus. This abnormal projection might provide excitatory input to regions which in turn project to the cerebral cortex, thus providing an alternate route of information transfer to the contralateral cerebral cortex.

The last proposed compensatory route is an increased use of existing ipsilateral projections, such as auditory and extralemniscal sensory pathways (Ettlinger, Blakemore, Milner, and Wilson, 1972). These are abundant in the immature brain and in cases of ACC may not regress or may undergo an extended period of plasticity (Jeeves, 1994; Jeeves, 1991; Windrem, DeBeur, and Finlay, 1988). This mechanism would also account for the fact that ACC patients exhibit deficits in speed and precision of performance on many tasks. Compelling evidence supports the view that the normal CC inhibits ipsilateral motor pathways from muscle groups involved in manual control (Thut, Halsband, Regard, Mayer, Leenders, and Landis, 1997) but is not involved in the facilitation of motor responses after contralateral movements (Meyer, Roericht, Einsiedel, Kruggel, and Weindl, 1995). Whereas some research indicates that motor-evoked potentials in response to cortical stimulation are not transmitted contralaterally via the CC (Misra and Kalita, 1995), others have suggested that the inhibition exerted by the intact CC prevents competition between crossed and uncrossed motor fibres, and is implicated in the coordination of symmetrical or precisely timed sequential movements (Schnitzler, Kessler, and Benecke, 1996). In other words, in ACC ipsilateral and contralateral motor projections may compete, resulting in impaired coordination due to poor coding of stimulus topography (Jeeves, 1992; Dennis, 1976), and exact timing of bilateral movements may be impossible.

The most parsimonious explanation for the observed pattern of deficits in ACC is that several of the above compensatory mechanisms are used simultaneously. Whereas visual, auditory, and language information may be transferred via the AC and subcortical pathways, at least one expert has suggested that there is no non-callosal mechanism for interhemispheric transfer between frontal and posterior parietal areas, which are involved in motor and spatial control (Lassonde, 1994). This would necessitate the use of ipsilateral projections for motor coordination and a consequent impairment on highly complex or speeded tasks. In split brain patients, there is no evidence for the use of ipsilateral projections, and therefore a similar impairment pattern would not be expected.

The major drawback to investigating the pattern of motor impairment in human patients is the relative infrequency of patients with pure ACC. We therefore decided to test manual coordination in samples of acallosal and callosotomized mice. Most of the literature about paw use in acallosal mice thus far has described the degree of relationship between paw lateralization and CC defects. While I/Ln mice, which never have a CC, are significantly more ambidextrous than normal mice (Gruber, Waanders, Collins, Wolfer, & Lipp, 1991), research on hybrids derived from C57 by I/Ln backcrosses has not found a relationship between ACC and ambidextrosity (Lipp, Bechel, Wolfer, & Scheffran, 1991). BALB/c mice, which have a lower degree of inbreeding than I/Ln, also failed to exhibit a relationship between CC area and degree of paw preference or handedness (Schmidt, Manhaes, & de Moraes, 1991; Bulman-Fleming, Wainwright, & Collins, 1992).

By comparison, there are relatively few studies concerning the relationship between CC area and manual coordination in mice. One study found no difference between acallosal, partially acallosal, and normal mice on a battery of bimanual tasks (Dufresne, unpublished manuscript). However, because subjects in this study were BALB/cWah1 mice, which have a low penetrance of the CC defect, the majority of subjects tested had a normal or only marginally small CC, which may mean that the apparent lack of deficits in mice with abnormal CC was due to low statistical power. Others (Lipp and Wahlsten, 1992) have reported a clear relationship between a decrease in CC area and an increase in errors on a manual coordination task in mice. However, with the development of a model for agenesis of the CC in mice (Wahlsten and Schalomon, 1994), which made large numbers of acallosal mice available for research, and with the development of a surgical technique for split brain surgery in mice (Schalomon and Wahlsten, 1995), the present comprehensive study of manual coordination in callosotomized and acallosal mice could be undertaken. It shows that while the inbred mouse strains which may exhibit the congenital CC defect are generally inferior in motor coordination to the hybrid mice used for callosotomy surgery, there is a significant impairment in motor coordination in all mice with callosal pathology, which is strongest in the case of congenital defects.

4.1. Experiment 1: Running Wheels

In this experiment, mice were tested on a computerized running wheel. This task relies on the subjects' inherent motivation to run for long periods and at high speeds. Rats will voluntarily run about 600 metres per hour (Gawley, Timberlake, and Lucas, 1987) and mice run 3 to 5 kilometres per day (Van den Pol, Cao, and Heller, 1998) when given free access to a running wheel. Based on such data, it has been suggested that wheel running elicits a distinguishable motivational state, which may be mediated by a different neural substrate than motivational states associated with consummatory responses (Timberlake and Lucas, 1989). This indicates that wheel running is an intrinsically reinforcing response in rodents.

An earlier study found no relationship between hereditary absence of the CC and wheel running behaviour (Bishop, Kruyer, and Wahlsten, 1996). In this study, we additionally tested callosotomized subjects and used a running wheel with an irregular pattern of rungs. This increased the task difficulty compared to the previous study, and the test may consequently be more sensitive to deficits in the acallosal group. In addition, mice were tested over several days, because inbred strains of mice have been shown to have differences in circadian period and therefore differ in their patterns of wheel running within a day (Possidente and Stephan, 1988). By testing average running over several days, differences in daily activity patterns would not affect the outcome of behavioural testing.

If CC defects do indeed affect motor coordination on this task, an impairment on several running behaviours would be expected. Insufficiently coordinated subjects should run at lower maximum and average speeds than unimpaired subjects, and stumbling during running would result on average in shorter uninterrupted bouts of running than would be expected from normal mice.

4.1.1. Materials and Methods

4.1.1.1. Subjects

A total of 110 mice between the ages of 48 and 87 days was used. This age range was chosen because by the age of 45 days the mouse CC is almost completely matured, with little additional myelination occurring during adulthood (Sturrock, 1980). None of the subjects had previous experience on a running wheel, or had ever been used for any type of behavioural testing. Seventy-three subjects were B6D2F$_2$ hybrid crosses between the inbred strains C57BL/6J and DBA/2J, which always have a normal CC. Of these, 47 underwent surgical transections of the CC. Subjects were anaesthetized with isoflurane and a small hook-shaped knife was inserted through a single trephine hole dorsal to the posterior end of the CC and lateral to the midline. Three cuts at different angles were made by inserting the knife just dorsal to the CC and slightly lateral to the midline to avoid damage to the anterior cerebral artery or superior sagittal sinus. The angle of the knife was then changed such that the knife would be pressed onto the dorsal surface of the CC before withdrawing. Thus, the hook-shaped end of the knife transected the callosal fibres mainly during withdrawal of the knife. For sham surgeries, which resulted in a far lesser degree of callosal transection, the same procedure was followed, except that the knife was not pressed dorsalward before withdrawing (for further details, see Chapter 2, and see Schalomon and Wahlsten, 1994). In the present experiment, 29 subjects underwent the complete surgical procedure, while 18 underwent the sham procedure, with half of each group having surgery in the right hemisphere and half in the left. Subjects were given a minimum of two weeks to recuperate from surgery before behavioural testing.

The remainder of the subjects were comprised of between four and eight subjects from each of five recombinant inbred (RI) lines. These recombinant lines were derived from the F$_2$ cross of 129/ReJ and BALB/cWah1 parents and had been inbred by full-sib mating for between four and eight generations at the time of this study (Wahlsten and Schalomon, 1994). Thus, the RI mice were not highly inbred, not all their genetic characteristics may have been fixed at the time, and fertility was still reasonably high.

Some of the RI animals had a normal corpus callosum, whereas others had a small or completely absent corpus callosum. One of the strains (Line 11) was always normal with respect to CC anatomy, two were always abnormal (Lines 20 and 23), and two had a wide range of CC sizes (Lines 30 and 38). The numbering of these RI lines was established at the time the strains were first inbred and was entirely arbitrary.

4.1.1.2. Behavioural Apparatus

The basic behavioural apparatus has been described in detail elsewhere (Wahlsten, Bishop, and Kruyer, 1997). It consisted of a running wheel mounted on one inner wall of a 32.0 x 32.0 cm Acrylite box with a height of 30.5 cm. Attached to the outside of the wall opposite the running wheel was a metal nestbox (15.0 x 12.5 cm, 10.5 cm high) connected to the inside of the Acrylite box by a hole with a diameter of 4.5 cm. The nestbox contained Aspen chip bedding, one compacted paper "Nestlet" for nest building, and the spout of a water bottle. The floor of the Acrylite box was covered with brown packing paper with wax paper backing, and held three food blocks (Wayne Rodent Blox 8604), which were supplemented daily as needed. The wheel used in this experiment differed from that described previously (Wahlsten, Bishop, and Kruyer, 1997). It was a "Fritz Plastic Playwheel", made of orange or brown plastic, with a front inside diameter of 13.6 cm, tapering to a back inside diameter of 12.8 cm. The wheel was 6.5 cm wide and originally had 36 flat rungs (4 mm wide), spaced 7 mm apart. Of these a total of 18 rungs had been removed at the rim, so that the pattern of remaining (*r*) to missing (*m*) rungs was

1*r*, 1*m*, 2*r*, 2*m*, repeated 6 times. As a result, the running wheel had a number of unevenly spaced gaps of up to 29 mm width, which subjects were forced to step across when running on the wheel (see Figure 4.1.1). Outside the box, attached to the wheel's axle, was a black disk (diameter 12.0 cm), which held three reflective strips covering 20 degrees of arc each and placed 120 degrees apart at different distances from the axle. Three photo cells attached adjacent to the disk detected the strips when the wheel was turning, and enabled the observation of partial turns.

Movement of the wheel as detected by the photo cells was recorded by programs written in the C programming language on a 486-DX33 computer. The program could have been written to collect data concerning the direction of wheel movement, which could presumably have provided insight into the direction of approach to the wheel by the subjects. However, the program as used in the current experiment did not collect such data. For the purpose of this experiment, any movement of the wheel such that the same reflective strip was sensed twice consecutively was defined as a swing, a measure of playing rather than running. Actual running was defined as movement of two different strips past the photo cells within 0.75 seconds (Wahlsten, Bishop, and Kruyer, 1997). When time periods greater than this passed between detection of adjacent strips, a bout of running was defined as having ceased. The C program monitored four wheels simultaneously and recorded the number of swings, the number of 1/3 rotations, and cumulative time spent running during the current test period. The program also calculated the minimum time between detection of adjacent strips (maximum speed), modal rotation time, and the variability of rotation times (average squared difference between times for

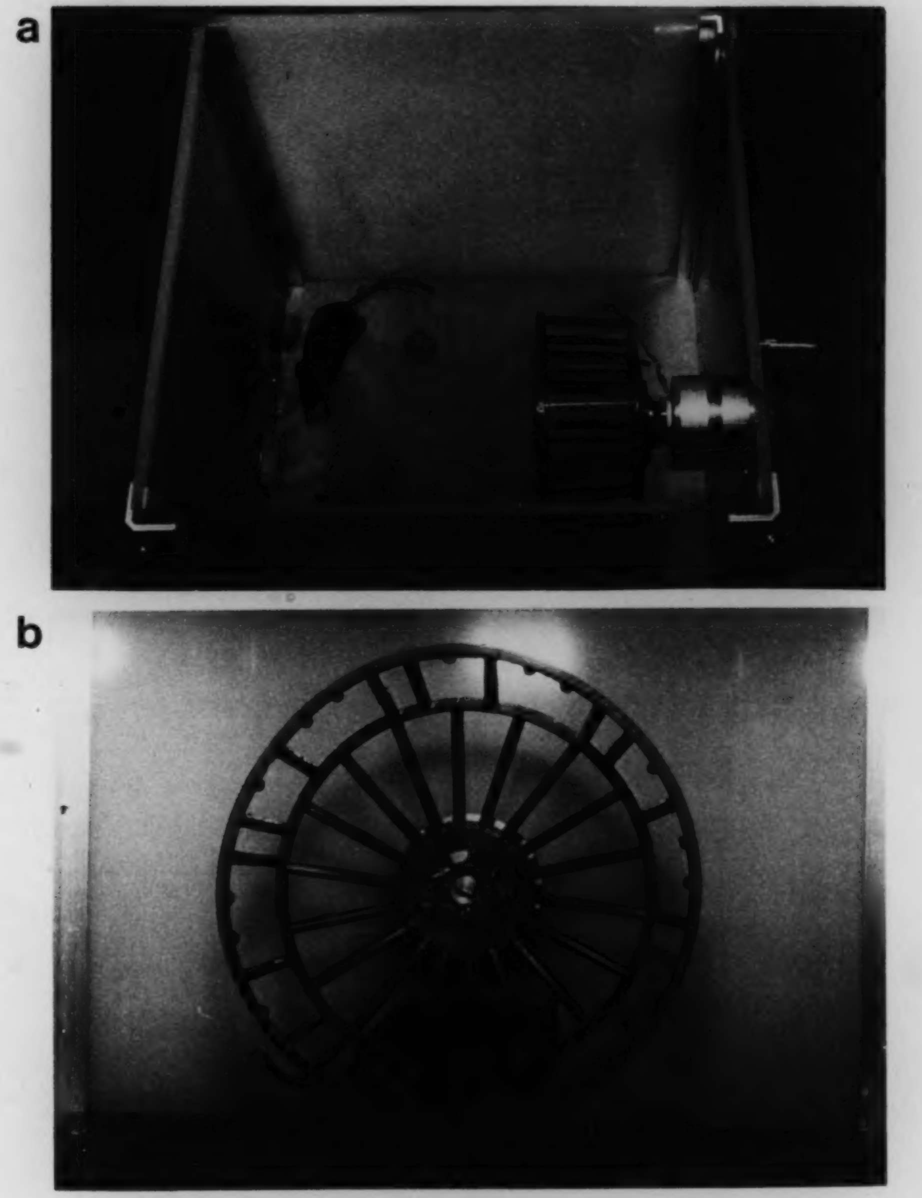

Figure 4.1.1. Running wheel apparatus used during behavioural testing showing (a) view of entire apparatus from above and (b) adapted wheel with missing rungs.

consecutive thirds of a rotation within a bout). Because mice run in bouts of high activity interspersed with periods of rest, a frequency distribution of the number of bouts of different lengths was also compiled, the numbers of bouts less than 1 second in duration, from 1 to 9 seconds in duration, and 10 or more seconds in duration were counted, and the longest bout length (to a maximum of 100 seconds) was recorded. Subjects of all groups were randomly assigned to one of the four running wheels and any one of the numerous five-day test periods of data collection such that any group of four mice tested simultaneously may have consisted of subjects from one or several different experimental groups.

4.1.1.3. Behavioural Testing and Histology

B6D2F$_2$ mice were randomly assigned to control, surgery, and sham surgery groups. RI mice were chosen randomly from larger populations of mice of the appropriate line and age. Due to the fact that the day-night schedule in the main mouse colony room differed from that in the testing room, mice in individual home cages were brought into the testing room at least three days before the start of testing. The light schedule in the main colony room was a 12 hours light/12 hours dark cycle, with lights off at 6 pm. The light schedule in the testing room was also a 12 hours light/12 hours dark cycle, however, lights were switched on six hours earlier, at 12:00 midnight. Past research has shown that exposing mice even to brief flashes of light during the late part of the dark phase can advance onset of running wheel activity periods by four hours within one day

(Van Den Pol, Cao, and Heller, 1998). Therefore, the three day adjustment period should have been sufficient to achieve a six-hour shift in activity patterns.

On day 1 of testing at 12:00 noon, mice were weighed and individually placed at random into one of the four test boxes, where they remained for the following five test days. Data were collected for 22 hours per day, from noon to 10 am. Therefore, each testing period began with the dark phase to take advantage of the fact that mice and rats are most active during the early part of each dark phase (Buettner, 1991; Pirke, Broocks, Wilckens, Marquard, and Schweiger, 1993). Food and water were provided *ad lib* and were checked by the experimenter every day between 10 am and noon . In order to minimize disturbance to the subjects, the room was never entered outside this daily 2-hour period, and the only background noise in the room was that made by the computer and ambient noise from the building. At the end of the five day test period, subjects were removed from the test boxes and weighed again. Before the next group of subjects was tested, the entire running wheel apparatus and nest boxes were thoroughly cleaned with alcohol followed by soapy water and finally rinsed with pure water. The metal parts of the wheel axle extending into the testing cubicle were cleaned with alcohol only.

Within 24 hours, subjects were anaesthetized with an overdose of pentobarbital (120 mg/kg IP), and perfused intracardially with approximately 10 ml of normal saline

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Footnote:

Approximately 20% of this data was collected by Deena McDougall, the remainder by the author of this thesis.

followed by 20 ml of neutral 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were immediately extracted from the skull and placed in fresh fixative for a minimum of one week. At that time they were blotted dry, blocked to a standard configuration (Wahlsten, 1984), and weighed. Brains of RI mice and control B6D2F_2 were bisected midsagittally. The left half of each brain was stained *en bloc* using gold chloride (Schmued, 1990). Midsagittal CC areas were measured using the JAVA video analysis system from Jandel Scientific, with special attention being paid to excluding the dorsal commissure of the fornix and superior fornix from the measurement (see Figure 4.1.2). Because this histological technique did not permit analysis of the extent of surgical damage, the brains of subjects in the surgery and sham surgery groups were sectioned coronally throughout the entire rostrocaudal extent of the CC. Sections were cut on a freezing microtome at 60 microns thickness and every fourth section was mounted on gelatin coated slides, stained with gold chloride (Schmued, 1990) and permanently coverslipped. To estimate the area of undamaged CC fibres, the percentage of non-transected CC fibres was estimated in each section in which the CC was crossing the midline, averaged over sections, and multiplied by the mean CC area of control B6D2F_2 subjects, which was 0.8262 mm^2. This was considered the best available method of estimating CC area from the available histology; however, it must be remembered that this method automatically reduced the variance of CC areas in those subjects with incomplete transection of the CC.

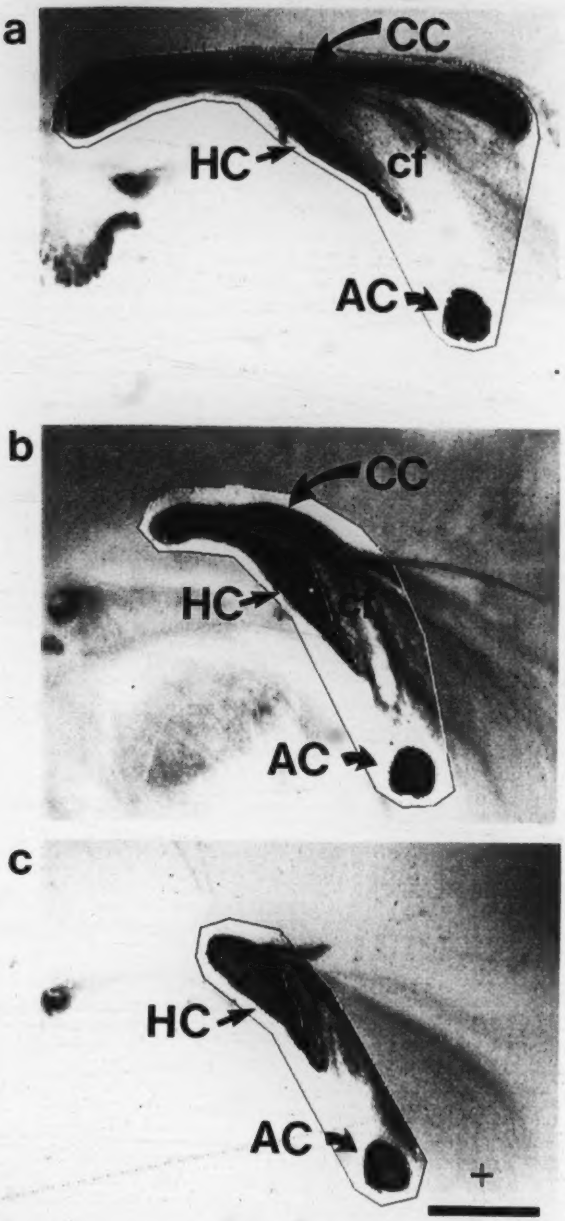

Figure 4.1.2. Video printer images analysed using JAVA software. Shown are midsagittal views of (a) normal brain, (b) partially acallosal brain, and (c) completely acallosal brain. Areas of commissures are outlined. Note that image obtained from the printout lacks detail visible during video analysis. Abbreviations: CC - corpus callosum, HC - hippocampal commissure, AC - anterior commissure, cf - columns of fornix. Scale bar = 1 mm.

4.1.2. Results

Preliminary analysis of data showed that running wheel behaviour did not differ significantly between pigmented mice (all B6D2F_2 mice as well as many of the RI mice) and albino mice. Therefore, because the differences in visual acuity between pigmented and non-pigmented mice did not appear to affect behaviour, coat colour of subjects was not used in further statistical analyses.

For several of the analyses, subjects were subdivided into four groups, normal and abnormal B6D2F_2 subjects, and normal and abnormal RI mice. Preliminary one-way ANOVAs showed that control B6D2F_2 subjects differed significantly ($p<.005$) from B6D2F_2 subjects that had undergone sham surgery on only one dependent measure, the mean minimum rotation speed, which was somewhat lower in control subjects (161 versus 196 msec/ 1/3 rotations). The most likely cause for this difference was the fact that sham surgery mice had smaller CC areas than control B6D2F_2 mice, because the only sham surgery subject that had a completely normal CC area had a mean minimum rotation speed of 17. In other words, the small difference between control and sham B6D2F_2 mice was apparently due to CC damage, surgery in itself caused no behavioural effects. Due to this fact, sham surgery subjects were treated not as a separate experimental group for the purpose of analysis, but were assigned into groups based on their strain (B6D2F_2) and on CC size. By using this approach, the number of experimental groups defined for statistical analyses was decreased, which increases the power of statistical tests.

CC area measures in the entire sample had a bimodal distribution, with modes around 0.00 and 0.85 mm^2 (see Figure 4.1.3). Normal mice were defined as having a CC

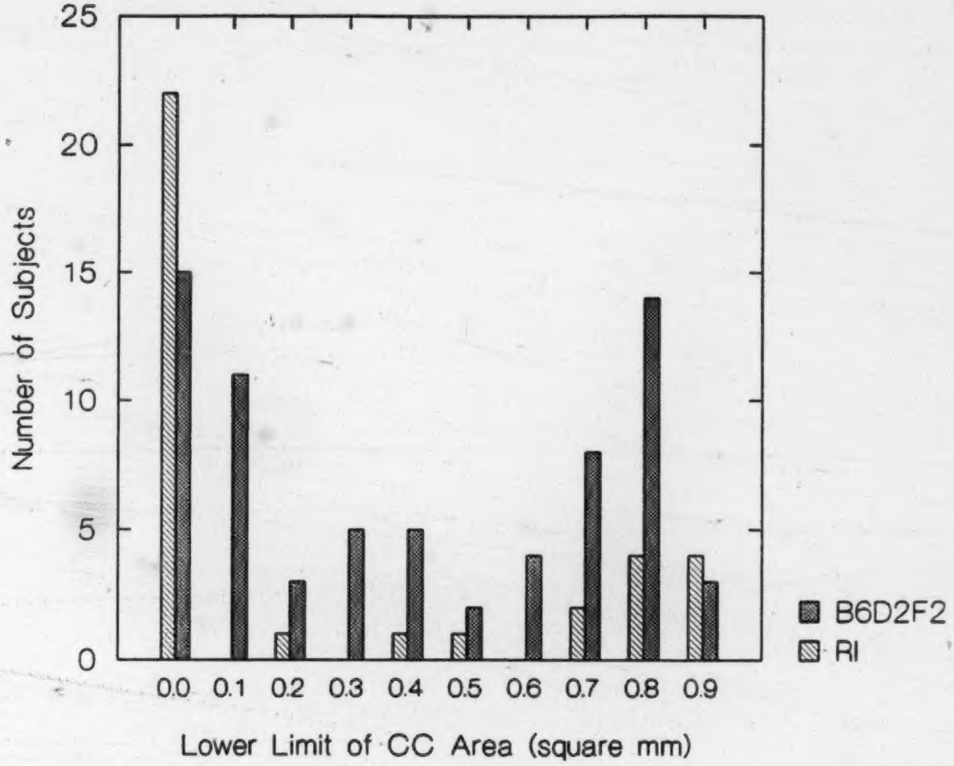

Figure 4.1.3. Distributions of CC areas in B6D2F_2 and RI mice. Shown is the lower limit of the CC area, such that a histogram bar for one area includes data for subjects with CC areas from the value indicated as the lower limit up to, but not including, the value listed as the next higher lower limit.

area equal to or greater than 0.7 mm^2. Few subjects in both B6D2F$_2$ and RI groups had CC areas around 0.7 mm^2; most of the subjects had CC areas either greater or substantially less than this value. The majority of subjects with CC areas only slightly below the cutoff value were B6D2F$_2$ mice that had had sham surgery. Cutoff values for distinguishing between normal and abnormal CC areas in previous research were about 0.55 mm^2 (Wahlsten and Schalomon, 1994; Wahlsten, Ozaki, and Livy, 1992). The cutoff for normality in the present study is therefore somewhat more conservative in that some subjects that might have been considered normal in other studies were designated as having a CC defect in the present data set, which made it less likely that abnormal subjects as a group would score significantly different from normal subjects. For regression analysis, CC area measurements were centred on the strain (B6D2F$_2$ or RI) mean by calculating the difference between actual CC area and mean CC area of the subject's strain (i.e. B6D2F$_2$ or RI). This removed any influence of strain differences in CC area from the data, such that the centred CC area of each subject was a measure of relative CC size (small or large compared to the *strain* average) rather than absolute CC size. In addition, because preliminary analysis showed that body weight of the subjects did not change significantly ($p>.05$) from the beginning of testing to removal from the running wheels, only the body weight at the outset of testing was used in all analyses.

Means of the 10 measures described above were calculated for each mouse based on data from test days 2 to 5 only. On the first test day the mice were engaged in much exploratory and playing behaviour, and their performance was often not a good indication of wheel running. Previous research (Bishop, Kruyer, and Wahlsten, 1996) has shown that bouts less than one second in length and swings are not indicators of running behaviour, and these measures were highest on day 1 of testing. Of 110 mice tested on the wheels, 107 mice ran for 5 or more minutes on at least three days. The three mice not conforming to these guidelines were dropped from the data set. They did not differ ($p>.05$) from the other mice with respect to sex, age, brain weight, or body weight. All three were completely acallosal mice from the same RI line (line 38), and thus did differ from the other mice with respect to line and CC area. Of the remaining 107 mice, 5 did not run more than five minutes on one or two test days. These did not differ from the other 102 mice with respect to age, sex, brain weight, body weight, line number, or CC area, and were retained in the data set. Data from the first test day were lost for four RI mice, but their treatment during and after testing differed in no respect from that of the other mice, and they were retained in the data set. Two mice were dropped due to being outliers. One surgery subject was an outlier on swings (studentized $t=11.05$, $p<.001$, Cook's distance=.483), and another surgery subject was an outlier on variance of speeds (studentized $t=117.27$, $p<.001$, Cook's distance=.821; the large size of this effect might indicate that there was a problem with the computer software or setup of the running wheel for this subject). Thus, 105 mice remained in the final data set.

To verify whether B6D2F$_2$ and RI mice were comparable with respect to sex, age, brain weight, centred CC area, and body weight, *t* tests were performed (see Table 4.1.1). Due to the centering procedure, which expressed CC areas in terms of difference scores from the strain mean, no strain differences in centred CC area was present; however, the standard deviations computed for the *t* test provide a comparison of variability of CC area

Table 4.1.1

Descriptive Statistics (Means and Standard Deviations) and *t* Tests for Strain Differences on Independent Measures

	B6D2F_2		RI			
	mean	SD	mean	SD	*t*	*p*
n	70		35			
Sex*	0.01	0.50	0.07	0.50	-0.55	0.58
Age	65.0	9.8	73.5	7.6	-4.46	<.001
Brain Weight	0.43	0.02	0.46	0.01	-11.19	<.001
CC Area	0.44	0.33	0.29	0.40	-2.11	0.04
Body Weight	22.3	3.3	23.1	2.7	-1.23	0.22

* sex coded +1 for males, -1 for females

in the two strains. There were significant differences between strains in ages and brain weights. The mean age for RI mice was slightly greater than that of B6D2F$_2$ mice (73.5 vs. 65.0 days, *t*=-4.4, *p*<.001). Mean brain weight of RI mice was also significantly greater than that of B6D2F$_2$ mice (.462 vs. .426 g, *t*=-11.2, *p*<.001); this confirms the earlier finding that strains derived from BALB mice consistently have heavier brains than other inbred mouse strains (Wahlsten, 1983), although the effect might also have been due to the greater age of BALB mice. In order to analyse the relationship between running wheel measures across groups (all subjects pooled), Pearson correlations between all ten behaviours were calculated (Table 4.1.2). Rotations and time running were very strongly correlated (*r*=.967) and are probably indicators of overall levels of activity. Both these measures were also strongly and positively correlated with bouts longer than 9 seconds and with longest bout length, indicating that animals that frequently had long bouts of running also ran a lot in general. There was no significant correlation (*r*=.052, *p*=1.00) between swings and bouts less than one second in length. Very short running bouts in this sample may have been due to tripping at the beginning of a bout, before the animals had become proficient at stepping on the irregularly spaced spokes of the wheel. On the other hand, bouts less than one second were also significantly, but negatively (*r*=-.639, *p*<.001) correlated with variability of speeds, the other measure which might be an indication of stumbling.

A repeated measures ANOVA was performed to test whether there were group differences in the change of behaviour over days (see Table 4.1.3). If there was no behavioural change over days or if the changes were basically linear, it would be more

Table 4.1.2

Correlations (Pearson *r*, with Bonferroni Adjusted Probabilities)
between Means of Running Wheel Measures

	Swings	Time Running	Minimum Rotation Time	Modal Rotation Time	Variance Rotation Times	Bouts <1 Second	Bouts 1-9 Seconds	Bouts >9 Seconds	Longest Bout
Rotations	.557**	.967**	-.431**	-.524**	.041	-.153	.524**	.887**	.696**
Swings		.542**	-.416*	-.456**	-.160	.052	.593**	.367	.175
Time Running			-.327	-.419**	.046	-.103	.607**	.897**	.699**
Minimum Rotation Time				.645**	.175	.150	-.070	-.340	-.402*
Modal Rotation Time					.186	.150	-.115	-.436**	-.451**
Variability of Rotation Times						-.639**	-.172	.193	.256
Bouts < 1 Second							.274	-.328	-.432**
Bouts 1-9 Seconds								.292	.025
Bouts > 9 Seconds									.816**

Notes:

* $p <= .01$
** $p <= .001$
Correlations were calculated on all subjects (groups pooled, n=105) with measures averaged over test days 2 to 5
Source of Bonferroni correction of *p* was (*p** number of correlations)

Table 4.1.3

Repeated Measures Analysis of the Effect of Days of Testing for Four Groups of Mice
Showing the Mean and Standard Deviation of Running Wheel Measures for Each Group on Each Day

		Day 2		Day 3		Day 4		Day 5				
Behaviour	Group	mean	SD	mean	SD	mean	SD	mean	SD	Effect	F	*p*
Rotations	normal B6D2F$_2$ (n=25)	9121	5156	12751	5857	15729	7417	18302	9330	Group	13.4	<.001
	abnormal B6D2F$_2$ (n=45)	10729	5045	13525	5858	15969	7076	18081	7713	Days	61.9	<.001
	normal RI (n=10)	5873	3510	7323	4188	9596	6904	9550	7181	Days* Group	2.7	.005
	abnormal RI (n=25)	4680	4602	5337	4762	7130	4913	9158	5192			
Swings	normal B6D2F$_2$	1616	1035	1670	1302	1586	1007	1440	819	Group	0.8	.490
	abnormal B6D2F$_2$	1781	1381	1558	906	1413	819	1230	607	Days	4.0	.008
	normal RI	1700	1224	1186	730	1377	1126	1126	808	Days* Group	1.0	.410
	abnormal RI	1279	790	1196	928	1361	1296	1166	462			
Time running (minutes)	normal B6D2F$_2$	169	84	215	84	242	93	269	108	Group	17.0	<.001
	abnormal B6D2F$_2$	214	83	246	81	266	93	290	94	Days	37.6	<.001
	normal RI	108	51	132	67	163	104	156	102	Days* Group	1.1	.361
	abnormal RI	98	91	103	83	138	80	171	83			
Minimum rotation time (msec/ 1/3 rotation)	normal B6D2F$_2$	171	69	157	63	151	62	146	58	Group	8.1	<.001
	abnormal B6D2F$_2$	218	25	205	26	195	27	186	28	Days	52.0	<.001
	normal RI	206	32	206	24	195	28	189	24	Days* Group	2.0	.036
	abnormal RI	232	65	242	89	210	64	197	52			
Modal rotation time (msec/ 1/3 rotation)	normal B6D2F$_2$	293	82	262	71	244	67	229	76	Group	8.7	<.001
	abnormal B6D2F$_2$	346	74	312	58	284	54	271	54	Days	25.4	<.001
	normal RI	294	30	289	35	274	44	262	37	Days* Group	1.4	.183
	abnormal RI	415	163	408	175	358	154	310	103			
Variability of rotation times (msec^2)	normal B6D2F$_2$	3365	917	3494	843	3579	856	3574	964	Group	2.8	.043
	abnormal B6D2F$_2$	3847	819	3854	814	3661	921	3691	965	Days	0.5	.581
	normal RI	3804	628	3826	782	3837	883	3645	1095	Days* Group	1.0	.455
	abnormal RI	2998	1574	3230	1363	3073	1381	3153	1406			
Bouts < 1 second	normal B6D2F$_2$	652	496	552	582	451	452	362	289	Group	2.9	.037
	abnormal B6D2F$_2$	911	1374	700	885	545	755	398	394	Days	2.5	.096
	normal RI	608	526	357	193	374	238	392	299	Days* Group	1.7	.088
	abnormal RI	1235	1721	1113	1655	1482	2244	1280	2314			
Bouts 1-9 seconds	normal B6D2F$_2$	1455	768	1360	790	1267	936	1141	655	Group	4.8	.004
	abnormal B6D2F$_2$	1867	1001	1822	977	1546	818	1310	670	Days	1.6	.199
	normal RI	1109	802	964	557	1115	684	911	442	Days* Group	5.0	<.001
	abnormal RI	902	753	848	678	1191	810	1303	377			

Rotations	normal B6D2F$_2$ (n=25)	9121	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	abnormal B6D2F$_2$ (n=45)	10729	5045	13525	5858	15969	7076	18081	7713	Days	61.9	<.001
	normal RI (n=10)	5873	3510	7323	4188	9596	6904	9550	7181	Days*	2.7	.005
	abnormal RI (n=25)	4680	4602	5337	4762	7130	4913	9158	5192	Group		
Swings	normal B6D2F$_2$	1616	1035	1670	1302	1586	1007	1440	819	Group	0.8	.490
	abnormal B6D2F$_2$	1781	1381	1558	906	1413	819	1230	607	Days	4.0	.008
	normal RI	1700	1224	1186	730	1377	1126	1126	808	Days*	1.0	.410
	abnormal RI	1279	790	1196	928	1361	1296	1166	462	Group		
Time running (minutes)	normal B6D2F$_2$	169	84	215	84	242	93	269	108	Group	17.0	<.001
	abnormal B6D2F$_2$	214	83	246	81	266	93	290	94	Days	37.6	<.001
	normal RI	108	51	132	67	163	104	156	102	Days*	1.1	.361
	abnormal RI	98	91	103	83	138	80	171	83	Group		
Minimum rotation time (msec/ 1/3 rotation)	normal B6D2F$_2$	171	69	157	63	151	62	146	58	Group	8.1	<.001
	abnormal B6D2F$_2$	218	25	205	26	195	27	186	28	Days	52.0	<.001
	normal RI	206	32	206	24	195	28	189	24	Days*	2.0	.036
	abnormal RI	232	65	242	89	210	64	197	52	Group		
Modal rotation time (msec/ 1/3 rotation)	normal B6D2F$_2$	293	82	262	71	244	67	229	76	Group	8.7	<.001
	abnormal B6D2F$_2$	346	74	312	58	284	54	271	54	Days	25.4	<.001
	normal RI	294	30	289	35	274	44	262	37	Days*	1.4	.183
	abnormal RI	415	163	408	175	358	154	310	103	Group		
Variability of rotation times (msec^2)	normal B6D2F$_2$	3365	917	3494	843	3579	856	3574	964	Group	2.8	.043
	abnormal B6D2F$_2$	3847	819	3854	814	3661	921	3691	965	Days	0.5	.581
	normal RI	3804	628	3826	782	3837	883	3645	1095	Days*	1.0	.455
	abnormal RI	2998	1574	3230	1363	3073	1381	3153	1406	Group		
Bouts < 1 second	normal B6D2F$_2$	652	496	552	582	451	452	362	289	Group	2.9	.037
	abnormal B6D2F$_2$	911	1374	700	885	545	755	398	394	Days	2.5	.096
	normal RI	608	526	357	193	374	238	392	299	Days*	1.7	.088
	abnormal RI	1235	1721	1113	1655	1482	2244	[illegible]280	2314	Group		
Bouts 1-9 seconds	normal B6D2F$_2$	1455	768	1360	790	1267	936	1141	655	Group	4.8	.004
	abnormal B6D2F$_2$	1867	1001	1822	977	1546	818	1310	670	Days	1.6	.199
	normal RI	1109	802	964	557	1115	684	911	442	Days*	5.0	<.001
	abnormal RI	902	753	848	678	1191	810	1303	877	Group		
Bouts > 9 seconds	normal B6D2F$_2$	255	174	384	191	440	183	505	202	Group	12.3	<.001
	abnormal B6D2F$_2$	309	166	399	177	479	189	540	178	Days	25.0	<.001
	normal RI	143	65	229	133	287	228	283	210	Days*	1.4	.172
	abnormal RI	129	170	222	472	185	180	258	185	Group		
Longest bout (seconds)	normal B6D2F$_2$	51	20	61	22	71	21	80	21	Group	9.6	<.001
	abnormal B6D2F$_2$	51	23	62	21	69	23	80	23	Days	49.8	<.001
	normal RI	41	12	49	14	58	16	59	27	Days*	0.7	.670
	abnormal RI	28	26	34	30	47	34	51	31	Group		

appropriate to use means over days for each behaviour in further analyses in order to reduce the number of statistical tests and the danger of false positive findings by a factor of four. For the ANOVA, subjects were subdivided into four groups (B6D2F$_2$ mice with normal and abnormal CC areas, and RI mice with normal and abnormal CC areas). Significant group differences (*p*<.01) were found for all running wheel measures except swings, variability of rotation times, and bouts <1 second (which were mentioned above as possible indicators of stumbling).

Inspection of the data indicated that B6D2F$_2$ mice ran more than RI mice and had higher maximum and higher relative numbers of longer bouts; RI mice also had higher modal rotation times (indicating lower running speed) than B6D2F$_2$ mice, with normal mice in both strains having lower modal rotation times, that is faster running, than subjects with CC abnormalities (Figure 4.1.4a). There was a significant (*p*<.01) effect of day of testing for all variables except variability of rotation times, bouts <1 second, and bouts 1-9 seconds. Mice in all groups became more proficient at running over time, with an increase in total number of bouts, speed, and relative length of bouts. The group by time interaction was significant (*p*<.01) for two running wheel measures, rotations and bouts 1-9 seconds. With respect to number of rotations, the two groups of B6D2F$_2$ mice performed very similarly and had a larger number of rotations with a steeper rate of increase over days than RI mice, indicating faster learning of the motor task. The increase over days in RI mice was less pronounced and, in addition, the abnormal RI mice had between 1000 and 2000 fewer rotations per day than normal RI mice. This shows that RI mice did not run as much or increase rotations over time as much as B6D2F$_2$ mice, an

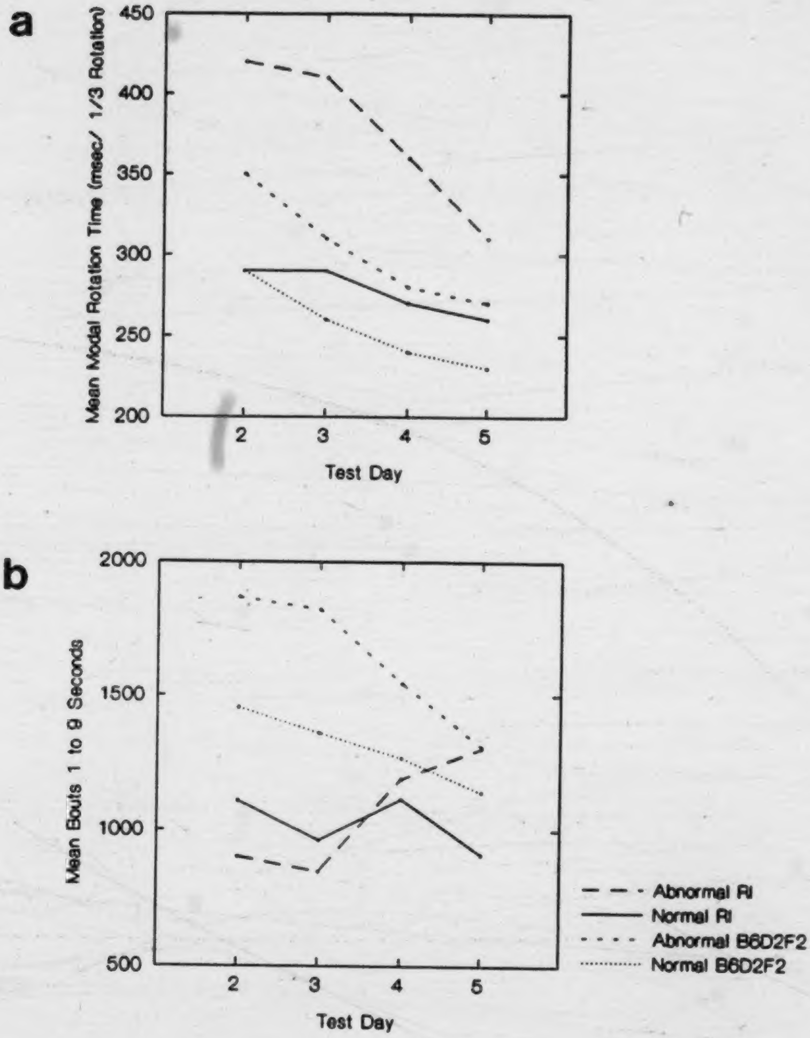

Figure 4.1.4 (a-b). Running wheel results for four groups of subjects on two behavioural measures, (a) modal rotation time, and (b) number of bouts 1 to 9 seconds in length.

effect which was more pronounced in RI mice with deficient CC. With respect to bouts 1-9 seconds in length, both normal and abnormal B6D2F2 mice showed a decrease over time, and abnormal B6D2F$_2$ mice always had a larger number of these intermediate length bouts than normal B6D2F$_2$ (Figure 4.1.4b). Normal RI mice did not have as many bouts 1-9 seconds in length as B6D2F$_2$ subjects and did not show a change over days. Abnormal RI mice initially had the lowest number of bouts 1-9 seconds in length of all four groups. However, abnormal RI mice were the only group that had an increase over time in bouts 1-9 seconds in length. All other groups had a decrease in short and intermediate length bouts over time, with a concurrent increase in bouts > 9 seconds, whereas abnormal RI mice had a decrease in the shortest bouts only, with an increase in intermediate length and long bouts. Unfortunately, modal bout length was not assessed, but mice with a congenital defect of the CC appeared to run less and for shorter bouts than normal littermates and far less than B6D2F$_2$ mice (whether normal or with surgical transection of the CC). In general, the change over days for running wheel measures was relatively smooth and linear for all four groups, justifying the use of means over days for all measures in further analyses.

Multiple regression analyses were performed to test whether any of the running behaviours could be predicted reliably by any of the independent measures or combinations of independent measures. For these analyses, the CC area of each subject was centred on its respective strain mean, abolishing any correlation of group and CC area. Effect coding was used for all nominal independent variables and the predictors used in the regression model were strain, sex, the strain by sex interaction, CC area, and the strain by CC area interaction. Regression analyses were performed stepwise backward, such that predictors that were not significant were excluded from the model one by one until all remaining predictors met the criteria for inclusion into the model. Because so many tests were done, the minimum significance for inclusion of any predictor into the regression model was set at p=.015, and the minimum tolerance for predictions was .98, so that only predictors that were highly independent of each other could be used simultaneously as predictors for the dependent measure. Additional analyses indicated that there were no good predictors that were excluded from the regression equation by this high tolerance value.

No significant predictors were found for variability of rotation times and bouts < 1 second (Table 4.1.4). For many of the remaining measures, both sex and strain were significant predictors, with B6D2F_2 mice generally performing better than RI mice. Centred CC area was a significant predictor for minimum and modal rotation times; both measures increased with decreasing CC area (see Table 4.1.5 and Figure 4.1.5). This indicates that mice with a greater number of CC fibres connecting the cerebral hemispheres had greater maximum speeds and generally ran faster.

The interaction of strain by centred CC area was significant (p=.015) only for bouts 1-9 seconds. As indicated above, B6D2F_2 mice had more bouts 1-9 seconds in length than RI mice, with mice with surgical transections of the CC having more bouts 1-9 than normal B6D2F_2s. There was no mean difference between normal and abnormal RI mice for bouts 1-9 seconds in length.

The finding that sex was a frequent predictor of running wheel behaviour was

Table 4.1.4

Descriptive Statistics (Means and Standard Deviations) and *F* Tests for Strain and Sex Differences on Means of Running Wheel Measures

	RI males		RI females		$B6D2F_2$ males		$B6D2F_2$ females			
	mean	SD	mean	SD	mean	SD	mean	SD	*F*	*p*
Rotations	5365	3847	8239	5051	11053	4154	17486	5854	28.1	<.001
Swings	938	362	1534	855	1273	675	1763	901	4.9	.003
Time Running (min)	107	64	149	79	201	56	283	78	29.4	<.001
Minimum Rotation Time (msec/ 1/3 rotation)	222	64	202	35	192	51	178	42	3.2	.027
Modal Rotation Time (msec/ 1/3 rotation)	387	147	308	68	308	66	267	47	8.3	<.001
Variability of Rotation Times ($msec^2$)	3636	1248	3054	1151	3845	957	350	554	93.1	.030
Bouts < 1 second	814	1424	1203	1708	563	956	617	370	1.7	<.001
Bouts 1-9 seconds	981	661	1103	501	1195	443	1824	859	9.5	<.001
Bouts > 9 seconds	146	124	257	232	357	125	477	152	18.1	<.001
Longest Bout	39	24	47	26	61	20	70	17	10.4	<.001

Table 4.1.5

Multiple Regression Analysis of the Means of Running Behaviours over Days 2 to 5 of Testing

	Regression Analysis of Variable Means			
Running Variable	Predictor(s)	Standardized Coefficient	p(2-tailed)	Adjusted R^2
Rotations	strain	.55	<.001	.428
	sex	.40	<.001	
Swings	sex	.32	.001	.092
Time running (minutes)	strain	.59	<.001	.445
	sex	.37	<.001	
Minimum rotation time	strain	-.28	.003	.150
	centred CC area	-.33	.001	
Modal rotation time	strain	-.35	<.001	.281
	sex	-.30	.001	
	centred CC area	-.34	<.001	
Variability of rotation times (msec^2)	---			
Bouts < 1 second	---			
Bouts 1-9 seconds	strain	.34	<.001	.217
	sex	. 31	.001	
	strain* centred CC area	-.22	.015	
Bouts > 9 seconds	strain	.53	<.001	.337
	sex	.30	<.001	
Longest bout (seconds)	strain	.45	<.001	
				.199

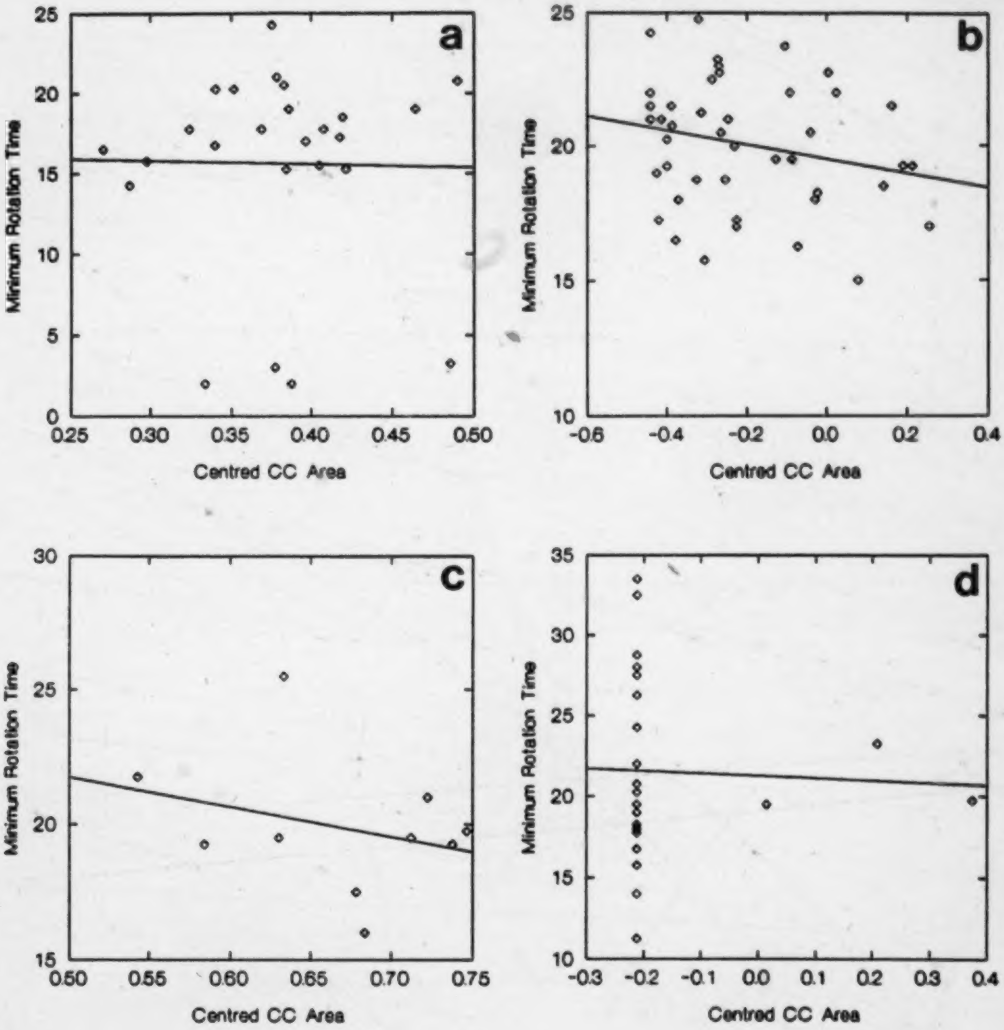

Figure 4.1.5 (a-d). Scatter plots of minimum rotation time (units: 3 rotations/ 10 seconds), which is a measure of maximum speed, against centred CC area for (a) normal B6D2F$_2$ subjects, (b) abnormal B6D2F$_2$ subjects, (c) normal RI subjects, and (d) abnormal RI subjects.

due to the fact that female mice generally ran much better (had more and longer bouts and higher modal speeds) than male subjects regardless of strain (see Table 4.1.4). However, this sex difference was smaller in size than the strain differences, such that B6D2F$_2$ males were still generally superior to RI females. Interestingly, similar sex differences were found in previous research (Bishop, Kruyer, and Wahlsten, 1996), and further research on this superiority of females is clearly warranted because females, which are usually thought to be more variable than males on most behavioural measures due to interference of estrus cycles with testing schedules, are often not used as test subjects.

In summary, the above experiment yielded several major findings. The strongest determining factor in running wheel performance was the strain (RI versus B6D2F$_2$) of the subject, with a minor superiority of females over males. CC deficits also affected performance on several measures. Mice with congenital defects of the CC did not improve performance over time or run as much or as well as any other group. Subjects with surgical CC section did not show as many or as clear deficits, but had relatively more intermediate length and relatively fewer long bouts than littermate controls.

4.2. Experiment 2: Balance Beam

A balance beam was used to further investigate motor coordination deficits in acallosal and callosotomized mice. Subjects were given the opportunity to explore a narrow, solid or notched bar, and hindfoot slips and falls off the bar were recorded. In order to motivate subjects to cross the bar, small nest boxes were attached to the far ends of the beam.. When a beam without nest boxes is used, mice will explore a beam but show far lower levels of activity (personal observation), with the present methodology, mice tend to shuttle back and forth between nest boxes at frequent intervals.

Previous research has shown that the completely acallosal I/Ln strain of mice makes more slips on this balance beam task than strains without CC defects (Lipp and Wahlsten, 1992). However, no correlation between CC area and slips was found within strains (Lipp and Wahlsten, 1992), and the behavioural deficit in the earlier study may have been due to strain differences unrelated to ACC. In the present experiment, the apparatus and testing procedure of the earlier study was therefore replicated using normal and CC transected B6D2F$_2$ mice and normal and acallosal RI mice of a number of different inbred lines with variable genetic background.

4.2.1. Materials and Methods

4.2.1.1. Subjects

A total of 155 mice was used, 101 of which were B6D2F$_2$ subjects. Of these, 33 were control subjects, 40 underwent CC transection surgery, and 28 sham surgery. The remaining 54 subjects were comprised of between 5 and 14 subjects from each of five RI strains (Line 11 with normal CC; Lines 19, 20, and 23 with absent CC; and Line 16 with varying degrees of CC abnormality). Three of these strains had also been used in Experiment 1.

4.2.1.2. Behavioural Apparatus

The apparatus used in this experiment was a narrow aluminum beam supported on two rods 66.0 cm above a table and parallel to a wall 50.0 cm behind the beam. Attached to each end of the beam was a clear plexiglass nestbox with open top, which contained a small amount of used bedding material from the subject's home cage. A schematic diagram of the beam giving dimensions is shown in Figure 4.2.1. The total length of the apparatus, including nest boxes, was 76.5 cm, and the depth of the notches on the beam was 1.5 cm. For testing on an easier, unnotched, solid surface, the beam was covered by a thin inverted-U-shaped metal strip covering the beam surface and sides. To make the beam less difficult to walk on, the notched or unnotched surface of the beam was covered with masking tape during testing (non-adhesive surface toward subject; for a representation of the surface structure see Figure 4.2.2). For the testing of albino animals only, the portion of the tape facing toward the video camera was coloured with a black marker just before testing to make foot slips more easily visible. The scent of the marker was probably detectable for these subjects; however, the marking itself was not visible to the subjects, and there was no significant correlation between albinism and any of the beam measures (r=-.256 to .142, p>.05). After testing each subject, the masking tape was discarded and the entire apparatus including nest box was cleaned with 50% ethanol to remove scent traces. At the end of each testing day the entire apparatus was immersed in soapy water, then thoroughly rinsed with pure water.

To record some aspects of behaviour, a video camera was used during testing. This camera was level with the beam at about 1 m distance from it opposite the back wall.

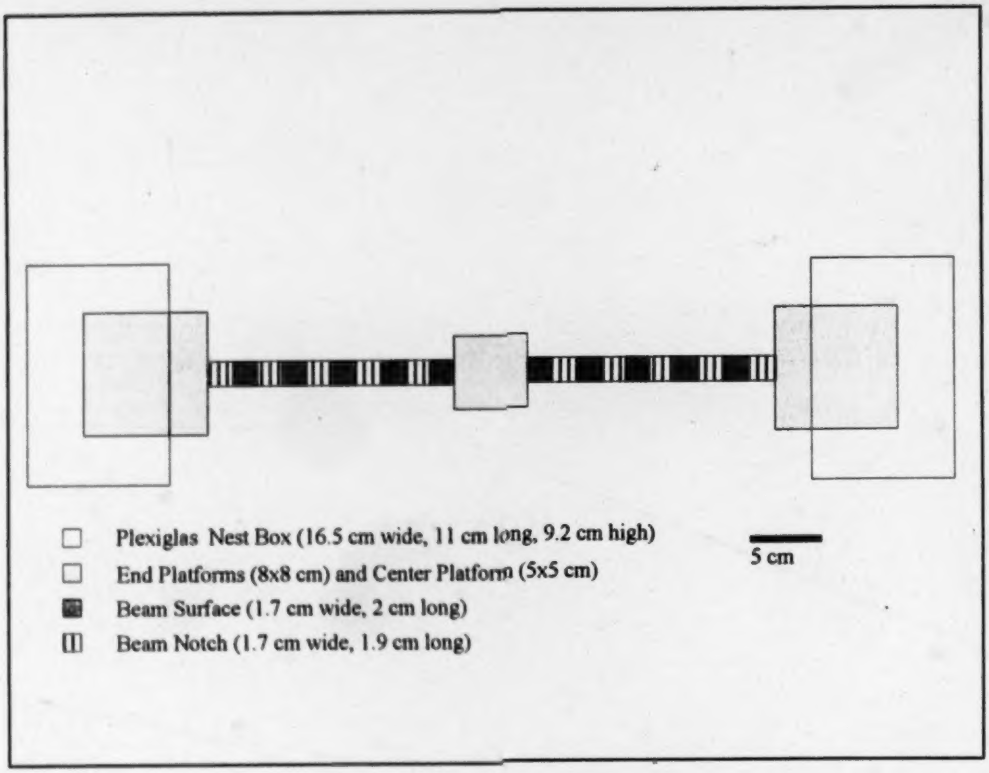
☐ Plexiglas Nest Box (16.5 cm wide, 11 cm long, 9.2 cm high)
☐ End Platforms (8x8 cm) and Center Platform (5x5 cm)
■ Beam Surface (1.7 cm wide, 2 cm long)
▥ Beam Notch (1.7 cm wide, 1.9 cm long)
5 cm

Figure 4.2.1 Schematic view of balance beam apparatus (view from above)

Figure 4.2.2. Surface texture of masking tape used to cover balance beam to prevent slipping (TESA brand).

Attached to this camera was a light with a red light bulb (7.5 Watts), to enhance video picture quality.

4.2.1.3. Behavioural Testing and Histology

Testing was performed in the same room and with the same light/dark cycle as in Experiment 1. Again subjects were housed in the testing room for at least three days prior to testing to adjust to the change in conditions. Subjects were weighed on test day 1. Testing on both test days was done during the first two hours of the dark period. To permit observation of behaviours, the fluorescent room lights were on during this period, but lights were dimmed to the lowest level that permitted the experimenter to see adequately (approximately 4.7 LUX). Due to the fact that one group of mice was always in the testing room for dark adaptation while the previous group was being tested, dim lights were on during the dark period of the three adaptation days as well as during testing itself.

On test day 1, the solid surface was used on the beam, whereas on test day 2 the notched surface was used. Subjects were set gently on the centre platform. They were then given five minutes in which to explore the entire apparatus (see Figure 4.2.3). If the subject had not made a minimum of three beam crosses during this period, it was given an additional two minutes on the apparatus. The experimenter interfered only if the animal fell off the beam or remained in one of the nest boxes for 30 consecutive seconds, in which case the subject was gently picked up and returned to the centre platform. The time from

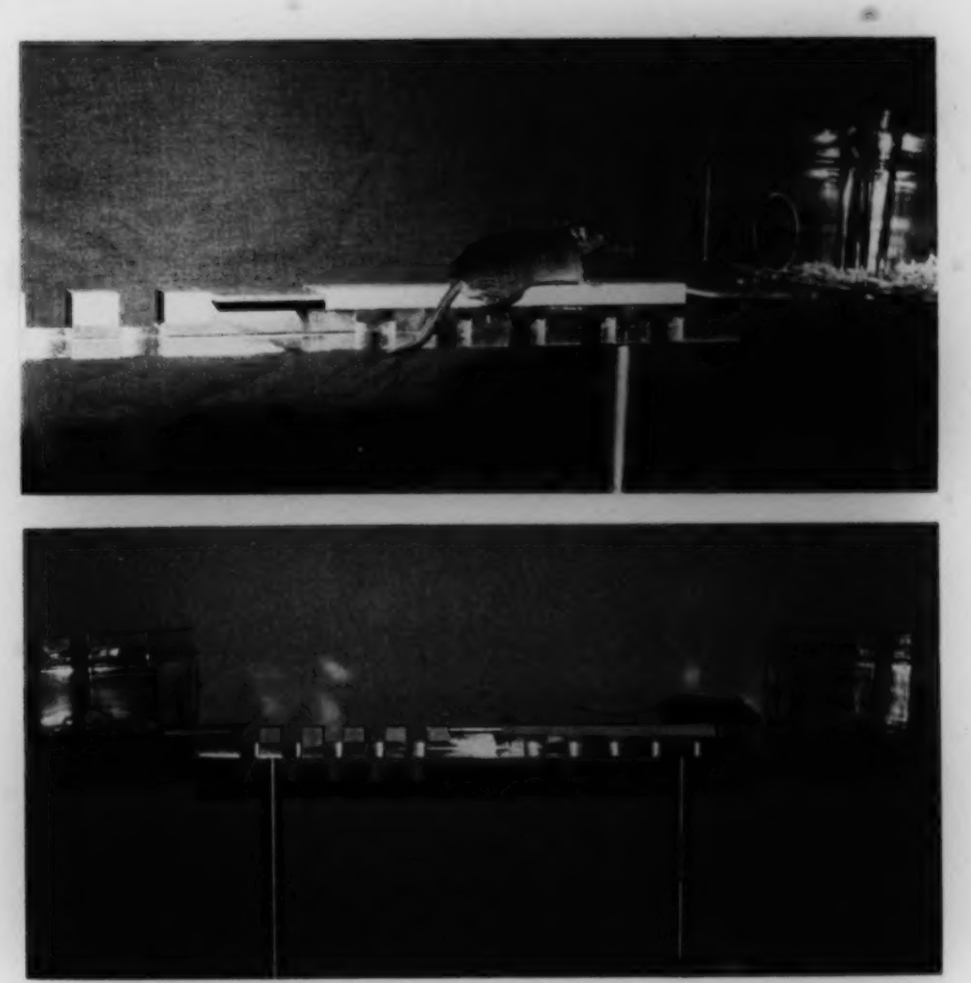

Figure 4.2.3. Mouse on balance beam apparatus. For purpose of illustration only, right half of the beam is covered with the U-shaped aluminium strip to provide a solid surface as used on Day1 of testing, and left half of the beam is uncovered and therefore notched as used on Day2 of testing. Nestboxes containing nesting material can be seen attached to far ends of beam..

falling off or pickup from the cage until placement on the platform was not included in the five minute time period. During the remainder of the test period, the experimenter was sitting at a distance of approximately three metres from the apparatus.

Several behaviours were scored by direct observation during testing. A cross was counted if the animal had crossed at least half the distance between the centre platform and one end platform (centre of mouse body, excluding tail, beyond midpoint of beam). If the animal turned around and walked back to the platform beyond this point, two crosses were counted. A cage entry was defined as movement of the mouse into the cage with at least half the body, excluding the tail, and a platform entry was defined as movement onto a platform with at least half the body. In addition, the number of times the subject was picked up from the table after falling off or picked up from the cage was recorded. The videotape recording, which could be reviewed frame by frame, was used to count the number of times the subject slipped off the beam surface with at least one hindfoot. Similarly, hindfoot slips off a platform were recorded. Forepaw slips were not recorded separately, as they were observed only in combination with hindpaw slips when the mouse was falling off the beam. Thirty-six subjects which had not made a minimum of three crosses on one of the test days were excluded from further testing and analysis and not processed histologically.

Within 24 hours of completing the second test day, subjects were perfused. Details of perfusion and histology were identical to those used in Experiment 1.

4.2.2. Results

A total of 119 mice performed to inclusion criteria. Performing and non-performing subjects did not differ with respect to strain or line number, sex, age, or body weight (p>.05). Because no perfusions were done on the 23% of subjects which did not perform to inclusion criteria, their CC areas cannot be compared to those of performing mice. However, mice of all types of lines were excluded on the basis of non-performance (3 of 30 control subjects, 10 of 30 surgery subjects, 7 of 28 sham subjects, 7 of 14 RI line 11 subjects, 2 of 13 RI line 16 subjects, 2 of 10 RI line 19 subjects, 1 of 9 RI line 20 subjects, and 4 of 8 RI line 23 subjects were excluded). It is therefore unlikely that CC area played a major role in determining whether or not a given subject performed to criterion in this experiment. Plots of each dependent measure for each test day by CC area were made and revealed no apparent outliers. Therefore all mice performing to criterion were retained in the data set.

As in Experiment 1, sham surgery subjects were not treated as a separate group for the purposes of statistical analyses, but pooled with surgery and control subjects on the basis of CC area. Of 28 sham surgery subjects, 21 performed to inclusion criteria. The average CC area of sham subjects was 0.530 mm^2, but only two had areas above 0.7 mm^2 and were pooled with control subjects. These two subjects did not differ significantly (p>.01) from controls on any of the dependent measures; undergoing the sham surgical procedure did not appear to cause any changes in motor coordination.

To check whether subjects of the two strains were comparable with respect to sex, age, brain weight, CC area and body weight, *t* tests were performed (Table 4.2.1). The only significant difference between the strains was for brain weight, which was greater in RI mice than in B6D2F$_2$ subjects (.467 versus .435 g, t=5.88, p<.001). This difference is typical of the two groups and is similar to that seen in the subjects in Experiment 1.

In order to analyse the relationship between beam behaviours on each test day, Pearson *r* correlations between all dependent measures were calculated. Because the main measure of overall activity, the number of beam crossings, was positively, although weakly, correlated with a number of the other behavioural measures (mean r=.156, computed by averaging correlation coefficients between beam crosses and six other measures over two days of testing), it was decided to correct these other measures for overall activity level. For this purpose, simple ratios of the raw measure divided by the number of beam crosses were calculated and used in all further analyses in place of the raw measures, and any further reference to these behavioural measures can be taken as referring to the measure after correction for activity.

Most beam measures were not strongly correlated with each other (see Table 4.2.2). Interestingly, the number of slips off the beam was significantly correlated with the number of crosses only on day 2 (r= -.419, p=.001). Personal visual observation indicated that this negative correlation is likely due to the fact that animals that walked less and instead froze or explored the beam were more likely to slip than animals that ran fast. The number of slips off the beam was significantly correlated (r=.520, p<.001) with slips off a platform only on day 1 of testing. Pickups from the table were significantly correlated only with slips off the beam and slips off a platform (r=.455, p<.001 for both)

Table 4.2.1

Descriptive Statistics (Means and Standard Deviations) and *t* Tests for Strain Differences on Independent Variables

	RI mice		$B6D2F_2$ mice			
	mean	SD	mean	SD	*t*	*p*
Number of Subjects	38		81			
Sex*	-.026	.50	-.031	.50	.046	.963
Age	71.0	3.8	61.8	7.2	.210	.834
Brain Weight	.467	.03	.435	.03	5.88	<.001
CC Area	.195	.34	.488	.33	-4.52	<.001
Body Weight	22.4	3.2	22.7	3.1	.550	.583

* coded +1 for males, -1 for females

Table 4.2.2

Correlations (Pearson *r*, with Bonferroni Adjusted Probabilities), between Beam Measures on Each Day

	Platform Entries[a]	Cage Entries[a]	Slips off the Beam[a]	Slips off a Platform[a]	Pickups from Table[a]	Pickups from Cage[a]
Day 1						
Crosses	-.302	-.499**	-.345	-.372	-.180	-.669**
Platform Entries[a]		.674**	.154	.183	.008	.068
Cage Entries[a]			.045	.117	.025	.439**
Slips off the Beam[a]				.520**	.455**	.173
Slips off a Platform[a]					.455**	.287
Pickups from Table[a]						.145
Day 2						
Crosses	.101	-.447**	-.419**	-.254	-.127	-.653**
Platform Entries[a]		.525**	-.149	-.118	-.115	-.089
Cage Entries[a]			.132	.134	.066	.704**
Slips off the Beam[a]				.121	.260	.301
Slips off a Platform[a]					.105	.355
Pickups from Table[a]						.187

Notes: * $p <= .01$
** $p <= .001$

[a] corrected for activity level
source for Bonferroni correction of *p* was (*p**number of correlations)

on day 1, when the easier, solid beam was used.

F tests were performed to test whether the four groups (abnormal and normal B6D2F$_2$ and RI mice, with some sham surgery subjects being designated as normal and some as abnormal, depending on remaining CC area) differed on any of the beam measures on either test day. The number of slips off the beam differed significantly (p<.001) between groups on both test days, because RI mice made far more slips off the beam than B6D2F$_2$ subjects. There was a marginally significant difference for the number of pickups from the table on day 1 (F=3.67, p=.014). This can be considered a measure of extreme slips, as it is always due to a slip off a beam with complete loss of balance. On this measure, normal RI mice had the lowest values and abnormal RI mice had the highest values, with B6D2F$_2$ mice being intermediate (see Table 4.2.3). The number of pickups from the cage on day 1 also differed significantly between groups (F=6.17, p=.001), with normal B6D2F$_2$ mice having the highest number of pickups and abnormal RI mice having the lowest number. Pickups from the cage can best be considered a measure of low activity, because highly active mice rarely remained in the cage for over 30 seconds. However, it is also a measure of exploratory behaviour, because mice that remained in the cages for longer periods rarely froze but rather climbed the cage walls.

In order to analyse whether behaviours on the solid beam on day 1 were related to behaviours on the notched beam on day 2, Pearson correlations between days were calculated (Table 4.2.4). Slips off the solid beam on day 1 and off the notched beam on day 2 were highly correlated (r=.629, p<.001), indicating that animals that had difficulties balancing on the notched beam had difficulties with the easier, solid beam as

Table 4.2.3

Descriptive Statistics (Means and Standard Deviations) and *F* Tests for Group Differences on Balance Beam Behaviours

	Normal B6D2F$_2$ (n=32)		Abnormal B6D2F$_2$ (n=49)		Normal RI (n=8)		Abnormal RI (n=30)			
	mean	SD	mean	SD	mean	SD	mean	SD	*F*	*p*
Day1										
Crosses	5.28	3.39	5.53	3.79	4.13	0.84	6.23	3.42	0.90	.444
Platform Entries*	1.62	0.44	1.58	0.52	1.64	0.30	1.62	0.56	0.06	.980
Cage Entries*	1.06	0.33	0.97	0.42	1.07	0.47	0.89	0.52	0.92	.432
Slips off the Beam*	0.72	0.46	0.60	0.54	1.60	0.84	1.45	1.13	11.10	<.001
Slips off a Platform*	0.46	0.35	0.35	0.33	0.54	0.54	0.45	0.45	1.09	.358
Pickups from Table*	0.09	0.13	0.05	0.10	0.03	0.07	0.15	0.19	3.67	.014
Pickups from Cage*	0.68	0.21	0.56	0.22	0.63	0.25	0.42	0.32	6.17	.001
Day2										
Crosses	8.63	4.98	9.61	5.21	6.50	3.46	9.70	6.92	0.92	.436
Platform Entries*	1.21	0.29	1.20	0.28	1.11	0.14	1.16	0.33	0.36	.779
Cage Entries*	0.63	0.26	0.55	0.29	0.49	0.40	0.53	0.29	0.89	.450
Slips off the Beam*	0.75	0.42	0.67	0.50	1.77	0.45	1.80	1.00	25.03	<.001
Slips off a Platform*	0.11	0.17	0.07	0.15	0.14	0.24	0.07	0.20	0.65	.583
Pickups from Table*	0.02	0.05	0.01	0.04	0.00	0.00	0.03	0.08	n.d.	
Pickups from Cage*	0.41	0.25	0.30	0.24	0.37	0.31	0.31	0.27	1.37	.254

n.d. = not defined due to lack of variance in one group
* corrected for activity level

Table 4.2.4

Correlations (Pearson *r*, with Bonferroni Adjusted Probabilities) for Beam Measures Between Days

	Crosses Day 1	Platform Entries Day 1	Cage Entries Day 1	Slips off the Beam Day 1	Slips off a Platform Day 1	Pickups from Table Day 1	Pickups from Cage Day 1
Day 2							
Crosses	.350*	.020	-.152	-.291	-.228	-.320	-.286
Platform Entries*	.101	.208	.088	-.052	.150	.057	-.118
Cage Entries*	-.127	.188	.320	.112	.269	.120	.124
Slips off the Beam*	-.144	.007	-.024	.629**	.277	.333	.030
Slips off a Platform*	-.133	-.156	-.011	.158	1.48	.080	.144
Pickups from Table*	.040	.051	.008	.000	-.010	-.102	-.134
Pickups from Cage*	-.276	.030	.252	.186	.236	.100	.271

Notes:
* $p <= .05$
** $p <= .001$
[a] corrected for activity level
Source for Bonferroni correction of *p* was (*p**number of correlations)

well. It is interesting to note that there was no correlation between pickups from the table on day 2 and slips off the beam on day 1 (*r*=.000, *p*=1.00) , which demonstrates that mice that had moderate problems on the solid beam did not necessarily have severe problems on the notched beam.

A repeated measures ANOVA over days was performed to investigate whether the performance of normal and abnormal B6D2F$_2$ and RI subjects changed over time (and thus, with increasing task difficulty and experience on the task). A series of one-way ANOVAs using effect coding showed that there were significant group differences on three beam measures (see Table 4.2.5). RI mice had significantly more slips off the beam than B6D2F$_2$ mice (*F*=22.58, *p*<.001). Abnormal RI mice had significantly more pickups from the table than the other three groups (*F*=5.54, *p*=.001). This indicates that mice with congenital CC abnormalities have more of the most severe coordination problems which result in falling off the beam. RI mice also had fewer pickups from the cage than B6D2F$_2$ mice (*F*=4.86, *p*=.003), indicating that B6D2F$_2$ mice less more active.

Behaviours changed over days on almost all measures. Contrary to previous predictions, mice made significantly more beam crosses on day 2 (*F*=28.09, *p*<.001). The number of slips off the beam (corrected for number of beam crosses) increased nonsignificantly from day 1 to day 2 (*F*=3.69, *p*=.057), indicating that the notched beam was at best marginally more difficult to cross than the solid beam. However, task difficulty was confounded with learning and mice had had previous experience with the general task on day 2, which might account for the relatively minor increase in slips.

Table 4.2.5
Repeated Measures Analysis of Variance of the Effect of Days of Testing
for Four Groups of Mice

Measure	Day	Normal B6D2F$_2$		Abnormal B6D2F$_2$		Normal RI		Abnormal RI		Group Effect		Day Effect		Group*Day Interaction	
		mean	SD	mean	SD	mean	SD	mean	SD	*F*	*p*	*F*	*p*	*F*	*p*
Crosses	1	5.28	3.39	5.53	3.79	4.13	0.84	6.23	3.42						
	2	8.63	4.98	9.61	5.21	6.50	3.46	9.70	6.92	1.24	.298	28.09	<.001	0.29	.832
Platform Entries*	1	8.28	4.97	7.88	3.71	6.88	2.36	9.73	5.78						
	2	10.53	7.11	11.49	6.74	7.13	3.68	11.80	9.54	0.06	.983	55.18	<.001	0.25	.858
Slips off the Beam*	1	3.31	2.02	2.53	1.87	6.13	2.30	6.87	4.09						
	2	5.59	3.00	4.88	2.56	11.13	5.72	13.70	6.09	22.58	<.001	3.69	.057	1.34	.273
Slips off a Platform*	1	1.94	1.22	1.49	1.33	2.00	1.77	2.23	1.70						
	2	0.75	0.92	0.41	0.73	0.75	1.04	0.50	0.86	1.36	.259	61.75	<.001	0.58	.632
Cage Entries*	1	4.97	1.53	4.51	1.64	4.50	2.51	4.80	2.88						
	2	4.78	2.21	4.51	2.32	2.63	2.26	4.50	2.83	1.14	.338	79.92	<.001	0.51	.678
Pickups from Table*	1	0.38	0.55	0.22	0.42	0.13	0.35	0.67	0.76						
	2	0.09	0.30	0.04	0.20	0.00	0.00	0.20	0.48	5.54	.001	13.01	<.001	1.59	.195
Pickups from Cage*	1	3.06	0.84	2.53	0.87	2.50	0.93	1.83	1.05						
	2	2.66	1.58	2.10	1.33	1.75	1.58	1.87	1.25	4.86	.003	39.04	<.001	1.78	.155

*corrected for activity level

Interestingly, the number of pickups from the table decreased from test day 1 to day 2 (F=13.01, p<.001), so mice did learn to catch slips before falling off the apparatus. Slips off a platform decreased (F=61.75, p<.001), which is likely due to the fact that mice spent more time crossing the beam and less time exploring the apparatus from the centre platform at the beginning of the test period. No significant group by day interaction effects were found.

Multiple regression analyses of beam measures with strain, sex, the strain by sex interaction, centred CC area, and the strain by CC area interaction as predictors were performed (Table 4.2.6). Because so many tests were done, the minimum significance for inclusion in the model was set at p=.015, and the minimum tolerance for these predictions was .98. Strain was a significant predictor (p<.001) for slips off the beam on both test days. On both days RI mice made far more slips than $B6D2F_2$ mice. Both strain and CC however, this effect was not large (adj. R^2=.125). Abnormal mice were picked up from the cage less often than normal mice and $B6D2F_2$ mice were picked up more frequently than RI mice. This shows that abnormal mice are less likely than normal mice to remain stationary in the cage (which measure may be indicative of either fear or low activity levels) and that RI mice are less likely to remain stationary than $B6D2F_2$ mice.

In summary, the findings in this experiment parallel those of Experiment 1. $B6D2F_2$ mice were generally superior to RI mice on the beam crossing task, having fewer slips and falls off the beam. The group with the most pronounced problems (as measured by slips and falls) was the group consisting of RI mice with CC deficits, indicating a clear motor coordination disadvantage of congenitally CC deficient subjects. By contrast,

Table 4.2.6

Multiple Regression Analysis of Balance Beam Behaviours on Each Test Day

Measure	Predictor(s)	Standardized Coefficient	*p* (2-tailed)	Adjusted R^2
Day 1 of Testing				
Crosses	---			
Platform Entries	---			
Cage Entries	---			
Slips off the Beam	strain	-.830	<.001	.213
Slips off a Platform	---			
Pickups from Cage	strain	.145	.003	.125
	centred CC area	.217	.002	
Pickups from Table	---			
Day 2 of Testing				
Crosses	---			
Platform Entries	---			
Cage Entries	---			
Slips off the Beam	strain	-1.09	<.001	.388
Slips off a Platform	---			
Pickups from Cage	---			
Pickups from Table	---			

surgical transection of the CC did not result in any significant deficits.

4.3. Experiment 3: Claw Length

Rodents control claw length by cutting the claw with their incisors while holding the digit in place (Whishaw, Kolb, Sutherland, and Becker, 1983). It has been shown that claw cutting in the rat requires integrity of the motor cortex. Ablations of the rat motor cortex result in increased claw length due to problems with motor control of movements including the chewing of claws, and not due to factors related to motivation, such as a decrease in the overall amount of grooming (Whishaw, Kolb, Sutherland, and Becker, 1983). Claw cutting can therefore be viewed as an indication of motor function, where a decrease in motor ability is related to an increase in nail length. Claw cutting is a largely unimanual task, because the legs whose digits are not being cut are necessary only to support the animal and are not directly involved in claw cutting. However, claw cutting also requires fine motor control and thus might be affected by CC defects. Claws of a large number of strains of mice with various degrees of CC defects were therefore collected and claw length was measured along the ventral surface of the nail. A decrease in manual coordination on this task would be expected to result in greater claw length. In addition, a decrease in manual coordination might lead to increases in the variability of claw lengths.

There are a number of other factors which could theoretically affect claw length and which were not controlled in the present study. There are at the present time no data available on the comparative growth rate of nails in the strains of mice used in the present study; obviously, mice with equal levels of claw cutting could have long nails due to a high growth rate. In addition, differences in the base activity levels of the subjects used here could account for some differences in claw length. Results from Experiments 1 and 2 reported in sections 1 and 2 of this chapter indicated that base activity levels tended to be higher in the hybrid B6D2F_2 mice than inbred RI mice. High levels of activity could be positively correlated with claw length, because highly active mice might spend less time grooming as opposed to other forms of activity. On the other hand, high levels of activity could also be negatively correlated with claw length, because active mice might wear down or tear their claws during locomotor activity or because they might engage in claw cutting more frequently as part of their overall high levels of activity.

4.3.1 Materials and Methods

4.3.1.1 Subjects

A total of 197 mice was used. Forty-four of these were B6D2F_2 mice, this group included 14 control animals, 15 animals that had undergone CC transection surgery, and 15 that had undergone sham surgery. The remaining 153 subjects were comprised of between 8 and 10 mice from each of 16 RI strains (Lines 11, 12, 28, and 42 with normal CC; Lines 7, 15, 19, 20, 22, and 23 with abnormal CC; and Lines 10, 16, 27, 30, 31, and 37 with variable CC sizes). Four of these strains had also been used in Experiment 1 and five in Experiment 2.

All of the $B6D2F_2$ mice used in this experiment had previously been used in either Experiment 1 or 2. Some of the RI mice had been used in Experiments 1 or 2 as well, but the vast majority had been used only for breeding in the mouse colony. All subjects were housed in standard cages with soft aspen chip bedding, which presumably would not wear down the claws.

4.3.1.2. Procedure and Histology

Tissue from all subjects was collected at the time of perfusion, either after completion of behavioural testing for Experiments 1 or 2, or after breeding. Perfusion was conducted as described in Experiment 1. After perfusion, the left hind foot of the subject was removed at the level of the distal tibia and fibula and stored in fresh fixative with the subject's brain. Previous research on claw cutting in rats (Whishaw, Kolb, Sutherland, and Becker, 1983) also examined cutting of hindpaw, rather than forepaw, claws. The cutting of hindpaw claws should be more difficult for the subject, because it requires the subject to balance on one hindpaw, while chewing on the claws of the other hindpaw. Therefore, in light of the fact that acallosal subjects have most deficits on complex tasks, the cutting of hindpaw claws should be a measure that is more sensitive to the effects of CC agenesis.

If there was any obvious damage to the foot or one of the claws, the right foot was used. Research on paw preference in rodents has generally used tasks that assessed the preference for forepaws (see for example Bulman-Fleming, Wainwright, and Collins, 1992); therefore, little data on hindfoot preference are available. Research in rats has indicated, however, that in normal rats, claws on both hind feet are generally cut on a daily basis by most rats (Whishaw, Kolb, Sutherland, and Becker, 1983). Therefore, it is likely that if some mice showed preferences for claw cutting on one foot, the direction of this preference would not be consistent within the large sample of subjects examined in the present study.

Within one month of perfusion, the subject's foot was blotted dry and the three middle toes were removed at the level of the tarsals. Video images of the lateral aspect of each toe were made and analysed with the JAVA Video Analysis System from Jandel Scientific. As in previous studies of claw length in various species (Whishaw et al, 1983; Taylor and Hurnik, 1994), the length of the claw was defined as the curvilinear distance along the dorsal aspect of the nail from the tip to the point of attachment of the cuticle (see Figure 4.3.1).

4.3.2. Results

Claw cutting was directly observed in numerous subjects. Mice appear to lift one hind paw to the jaw, then chew one claw at a time for several seconds. Claws therefore

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Footnote:

All claws were measured by Geneva Liu.

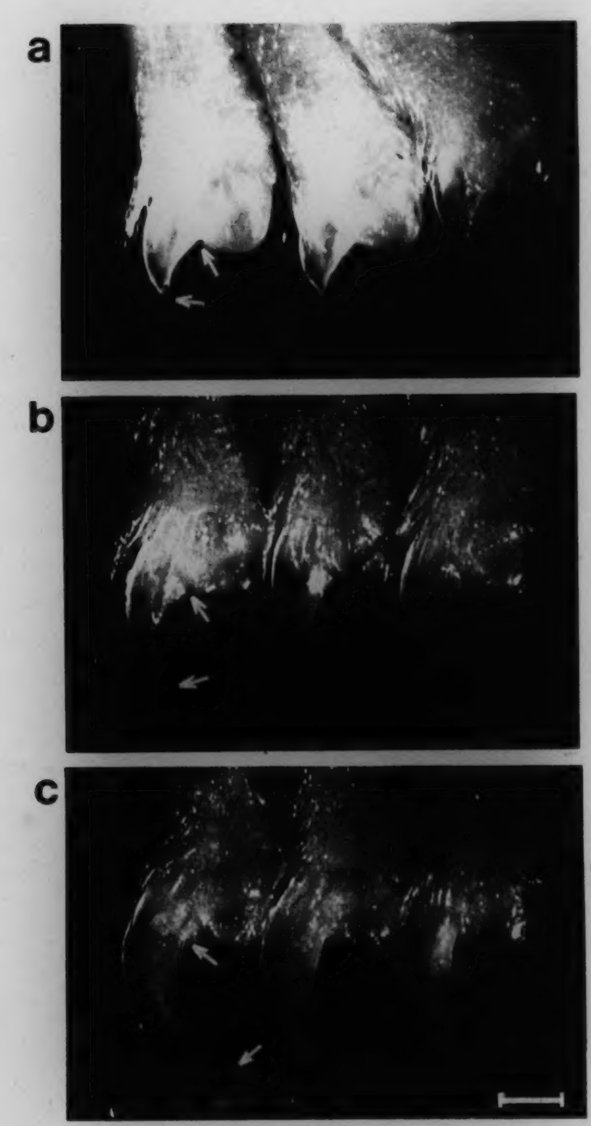

Figure 4.3.1 (a-c). Video printer images analysed using JAVA software. Shown are (a) very short, (b) normal length, and (c) abnormally long sets of three middle claws. Measuring line along dorsal aspect are indicated by arrows. Note that image obtained from printer lacks some detail visible during video analysis. Scale bar = 1 mm.

do not appear to be bitten off in one bite but with several smaller nibbles. During this time the mice balance on the other hind paw.

To calculate CC area of surgery and sham subjects, the percentage of non-transected CC fibres was multiplied by the mean CC area of control subjects which was 0.83 mm^2.

Of 197 mice in the original sample, 7 mice were dropped due to being outliers on the length of at least one claw (claw lengths of these subjects were 2.2 mm or greater). Three of these were normal RI mice, three were completely acallosal RI mice, and one was a $B6D2F_2$ control subject. Studentized *t* values for these animals varied between 2.658 and 9.556 ($p<.001$ for 195 df), Cook's distance between .035 and .387. Three of the excluded subjects were outliers on the length of more than one claw. Because the three claws were not measured in any specific order, statistical analyses were done using the average claw length as the dependent measure.

To test whether the strains used in this study were comparable with respect to sex, age, brain weight, centred CC area, body weight, and claw length, *t* tests were performed (Table 4.3.1). There were significant strain differences for age, brain weight, body weight, and claw length. RI mice were significantly ($t=7.11$, $p<.001$) older and more variable in age than $B6D2F_2$ mice, because they included many mice that had previously been used for breeding. As a consequence, RI mice were also significantly ($t=5.42$, $p<.001$) heavier than $B6D2F_2$ mice. Brain weight in RI mice was significantly greater than in $B6D2F_2$ mice, a difference similar in size to that observed in Experiments 1 and 2. There was a significant ($t=5.47$, $p<.001$) difference in claw length between

Table 4.3.1
Descriptive Statistics (Means and Standard Deviations) and *t* Tests for Strain Differences on Independent Variables and Claw Length

	RI mice		B6D2F$_2$ mice			
	mean	SD	mean	SD	*t*	*p*
Number of Subjects	147		43			
Sex*	-.044	.500	.081	.499	1.45	.149
Age	115.4	47.6	63.5	7.5	7.11	<.001
Brain Weight	.462	.026	.437	.029	5.49	<.001
CC Area	.368	.429	.430	.315	-0.88	.378
Body Weight	28.5	7.1	22.4	3.0	5.42	<.001
Claw Length	1.44	.253	1.23	.125	5.47	<.001

* coded +1 for males, -1 for females

the two strains. Claws of RI mice were generally longer and more variable in length than those of B6D2F$_2$ mice.

In order to analyse the relationship between independent measures and claw length, Pearson *r* correlations (with Bonferroni adjusted probabilities) between these measures were calculated (Table 4.3.2). Body weight and age were significantly (r=.637, p<.001) correlated, with older animals being heavier. Strain was significantly correlated with age (r=-.460, p<.001), with brain weight (r=-.371, p<.001), and with body weight (r=-.368, p<.001). As mentioned above, RI subjects were older and therefore heavier than B6D2F$_2$ mice and had the larger brain size typical of BALB-derived strains. Claw length was significantly correlated with strain (r=-.370, p<.001), RI mice having longer claws. It is important to note that claw length was not significantly correlated with age, body weight, or brain weight.

Differences between normal and abnormal RI and B6D2F$_2$ in the relationship between independent measures and claw length were analysed using Pearson *r* correlations (Table 4.3.3). Within B6D2F$_2$ subjects, claw length was not significantly correlated with any of the independent variables including centred CC area. Within RI subjects, claw length was significantly correlated with age (r=-.549, p<.001) and body weight (r=-.412, p=.040) in the group of normal RI mice, which indicates that older, heavier animals had shorter claws. No significant correlations between claw length and any independent variables were found for acallosal RI mice. Therefore, whereas normal RI mice become more proficient at claw cutting with increasing age, their acallosal littermates exhibit no significant improvement on this motor task. B6D2F$_2$ mice, which

Table 4.3.2
Overall Correlations (Pearson *r* with Bonferroni adjusted Probabilities) between Independent Variables and Average Nail Length

	Age	Brain Weight	Body Weight	Strain	Centred CC Area	Claw Length
Sex	.010	-.039	-.120	.105	-.047	-.088
Age		.250	.637**	-.460**	.140	-.079
Brain Weight			.219	-.371**	-.184	.238
Body Weight				-.368**	.217	-.114
Strain					-.006	-.370**
Centred CC Area						-.016

Notes: * $p <= .01$
** $p <= .001$
Source of Bonferroni adjustment of p is (p*number of correlations)

Table 4.3.3
Correlations (Pearson *r* with Bonferroni adjusted Probabilities)
between Independent Variables and Average Claw Length
for Normal and Abnormal B6D2F_2 and RI subjects

	Age	Brain Weight	Body Weight	Centred CC Area	Claw Length
B6D2F_2 subjects with normal CC (n=13)					
Sex	-.156	.188	-.772*	.031	-.310
Age		-.053	.481	.142	-.062
Brain Weight			.179	.561	-.221
Body Weight				.269	.199
Centred CC Area					-.277
B6D2F_2 subjects with abnormal CC (n=30)					
Sex	.272	-.187	-.817**	-.076	.121
Age		-.202	-.156	-.245	-.029
Brain Weight			.332	-.278	.286
Body Weight				-.282	.011
Centred CC Area					-.244
RI subjects with normal CC (n=51)					
Sex	.176	.044	.191	.111	.035
Age		-.043	.571**	.345	-.549**
Brain Weight			-.032	.121	.169
Body Weight				.306	-.412*
Centred CC Area					-.006
RI subjects with abnormal CC (n=96)					
Sex	.045	.014	-.094	-.030	-.119
Age		.235	.563**	-.081	-.191
Brain Weight			.199	-.242	.091
Body Weight				.074	-.252
Centred CC Area					-.041

Notes: * $p <= .05$
** $p <= .001$
Source of Bonferroni adjustment of *p* is (*p**number of correlations)

have significantly shorter claws (see Table 4.3.1), appear to be more proficient at claw cutting than the inbred RI mice, regardless of age and of whether they have a surgical CC defect.

A multiple regression analysis was performed to check whether claw length could be predicted to a significant degree by strain, sex, the strain by sex interaction, centred CC area, or the centred CC area by strain interaction. Minimum significance for inclusion in the model was set at p=.015 and tolerance at .98. The only significant predictor for claw length was strain (standardized coefficient=-.370, p<.001, adjusted R^2=.133), due to the fact that RI mice had significantly longer claws.

In summary, the above experiment yielded relatively little insight into the relationship between CC abnormality and claw length in mice. The most consistent finding was a difference in claw length between strains, with RI mice having significantly longer claws than hybrid $B6D2F_2$ mice regardless of CC status.

4.4. Discussion

We examined whether bimanual coordination of congenitally acallosal or surgically CC transected mice differed from that in normal mice of hybrid or recombinant, partially inbred strains.

Significant sex and strain differences were found on all behavioural tasks. On the wheel task, the performance of females in both groups was superior to that of males with respect to overall activity, bout length, and speed, despite the fact that the CC area in females was significantly smaller than in males. This sex difference in CC area, which has been reported previously (Zaidel and Aboitiz, 1995; Supprian and Kalus, 1996), is largely due to the fact that the average body size of females is smaller and due to the fact that body size is highly correlated with brain and CC sizes; it serves to illustrate the fact that having a somewhat smaller CC in itself does not cause problems with motor coordination.

On the claw cutting task, strain differences were also found, either because the hybrid mice are more proficient at claw cutting, or because of the overall higher levels of activity exhibited by the hybrids. It is comparatively less likely, but possible, that the strain difference in claw length was due to a higher growth rate of claws in the inbred strain; highly inbred lagomorphs, for example, may exhibit accelerated growth of the incisors and claws (personal observation). The only effect of CC area in the present data was observed in the RI strains of mice. Whereas young RI mice had comparatively long claws regardless of CC status, RI mice with normal CC areas had shorter claw lengths with increasing age, indicating that claw cutting could be learned by RI mice with normal CC, but not by acallosal RI subjects.

Of major interest in the present set of experiments, however, were differences between control mice and mice with congenital and acquired CC defects. Hybrid mice with surgical transections of some or all of the CC exhibited mild impairments on several measures. On the running wheel, these subjects had significantly more intermediate length bouts than normal littermates, indicating that they ran for relatively shorter bouts than control animals. As predicted, CC transected mice also exhibited somewhat lower running speeds than control B6D2F$_2$ subjects. During balance beam testing, on the other hand, there was no difference between the performances of normal and CC transected B6D2F$_2$ subjects on any measure related to slipping or falling off the beam. In addition, CC transected mice did not exhibit any deficits in claw cutting.

Inbred mice with callosal agenesis, by contrast, exhibited impairments in all three experiments. On the running wheel, all RI mice ran less than hybrid B6D2F$_2$ mice, but this effect was more pronounced in acallosal RI subjects. In particular, abnormal RI mice had very few long bouts of running and an increasing frequency over days of intermediate length bouts only. Acallosal RI mice also exhibited the lowest maximum and average running speeds of any group. On the balance beam, acallosal RI mice fell off the beam (an indication of severe coordination problems) more often than normal littermates or B6D2F$_2$ subjects. Claw length of RI mice, regardless of CC status, was greater and more variable than that of the hybrid strain. Interestingly, normal RI subjects appeared to improve on this task with age. Only acallosal RI mice were consistently impaired at claw cutting. However, if there was a similar deficit in claw cutting by the B6D2F$_2$ hybrid mice with CC transections, it might not have been detected due to the relatively short interval (between three and four weeks) between surgery and evaluation of claw length, which limited the time during which claws could grow after the onset of any possible deficit on claw cutting due to surgery. Mouse claws grow between 0.10 and 0.13 mm per day (Lavelle, 1968), resulting in a total growth of 2 to 3 mm in a three week interval, which is approximately twice the total claw length of hybrid subjects. It is therefore very unlikely that the limited time since surgery was responsible for findings in surgery subjects.

One explanation for the consistent finding of impairments of acallosal RI mice is the fact that task difficulty of the running wheel and balance beam tasks employed in the present research was intentionally high because deficits in human acallosal patients are found most consistently on tasks with high task demands. An earlier comparison of running wheel performance of both normal and acallosal RI mice failed to find any evidence of impairment of acallosal RI mice (Bishop, Kruyer, and Wahlsten , 1996). While the apparatus employed in this earlier research and the present study was almost identical, it differed in one crucial respect: The earlier study used a high precision aluminum running wheel, with rungs spaced closely together at equal distances, the present study employed a cheap plastic running wheel with unevenly spaced bars. Not only were rungs placed at variable distances from each other, the minimum distance between rungs was higher, and the wheel as a whole was less stable. This made running on the wheel without stumbling, especially at higher speeds, an especially difficult task. The balance beam task also was fairly difficult, requiring subjects to cross a narrow metal beam with relatively smooth surface, which additionally had deep notches on the second test day. The notches actually appeared to create little additional difficulty for subjects, which did not exhibit greater deficits on the second test day.

Overall, of the three tests employed in the present set of research, the running wheel task was most difficult and complex, the balance beam task was of intermediate difficulty, and claw cutting was least complex. In the light of previous findings in acallosal human subjects it is therefore hardly surprising that the relative deficits of acallosal subjects were greatest on the running wheel task and mildest on the claw cutting task. Apparently, increasing task difficulty exacerbates deficits in acallosal mice as it does in acallosal humans.

It is apparent that whereas both CC transected B6D2F$_2$ mice and congenitally acallosal RI mice have deficits on some measures of manual coordination, these deficits are both larger and more extensive in the congenitally affected group. The acallosal RI mice had deficits on every behavioural task, whereas deficits of CC transected B6D2F$_2$ mice were found only on the most complex task, wheel running. This clear effect of CC abnormality on running wheel performance has not been found in previous research which compared normal and acallosal RI mice (Bishop, Kruyer, and Wahlsten, 1996). The only significant difference in methodology between the two studies was the wheel used during testing; a regular metal wheel with closely, evenly spaced stepping bars was used in the earlier study and a plastic wheel with unevenly spaced bars and large gaps was used in the present experiment. Clearly, increasing task difficulty resulted in the finding of a motor deficit in the present sample. Compared to abnormal RI subjects used in the Bishop, Kruyer, and Wahlsten (1996) study, RI subjects in the present sample ran for fewer rotations, whereas B6D2F$_2$ subjects actually ran more. In addition, modal rotation time in abnormal RI subjects, but not in other groups in the present sample was far higher than in the earlier study, indicating a reduction in running speed with the difficult wheel task.

One further possible explanation for the lack of running on the wheel by subjects with CC defects would be that to these subjects the uneven, difficult wheel is less rewarding than to subjects with normal callosa. The decrease in running therefore would not be due to motor coordination deficits, but to a lack of running motivation. The only way to test whether the difficult wheel is less rewarding than a normal wheel would be to give subjects a choice task and assess the preference for a normal as compared to an uneven running wheel both in neurologically normal and CC deficient mice. If subjects with CC defects showed a stronger preference for the normal wheel than normal subjects, CC defects might be responsible for a decrease in reinforcement value of the difficult wheel. However, even in such a choice task, the increase in preference for the normal wheel could be due to motor coordination deficits which could lead to painful stumbling, and therefore aversion to the difficult wheel.

The difference between mice with congenital and the surgically induced conditions appears to be of a truly qualitative nature; only acallosal RI mice did not improve on the claw cutting task and were unable to acquire the ability to run frequently for long bouts on the wheel. One question not addressed by the present program of research was whether this difference in the pattern of deficits was truly due to the differential effects of having a congenital defect of the CC as compared to CC damage induced surgically after maturation of the brain and therefore during a period of relatively low functional plasticity of the central nervous system. Instead, it is possible that differences in the pattern of deficits between CC defective RI mice and CC transected $B6D2F_2$ mice are due to inherent strain differences which become apparent only in case of CC defects.

In the present series of experiments, RI mice with CC defects had greater deficits compared to normal mice within the same strain than CC transected mice, as well as exhibiting different patterns of deficits, which makes it more plausible that differences between abnormal RI and $B6D2F_2$ mice were due to the different mechanisms of CC defects in these strains rather than mere strain differences. To resolve conclusively which of these explanations is correct, however, future research should involve comparisons of congenital CC defects versus CC transection *within* a strain of mice such that strain differences and differences in type of CC abnormality are not confounded. This could only be achieved by comparisons between congenitally normal but CC transected RI mice and RI mice with congenital defects of the CC.

Nor is a CC defect the only factor influencing behaviour on motor coordination tasks in the present set of experiments. The hybrid B6D2F_2 strain of mice outperformed the inbred RI strains on virtually all measures regardless of CC status. Previous research has shown that even single gene mutations in inbred mouse strains can affect behaviour on complex motor tasks such as wheel running or maze learning (Oliverio and Messeri, 1973). Therefore, due to the fact that inbred lines of mice are statistically far more likely than hybrids to be homozygous recessive for alleles which reduce overall genetic fitness, they may express genetic characteristics affecting motor behaviour which are never expressed in hybrids. In addition, several BALB/c mouse substrains are highly timid in novel environments (Lasalle, Halley, and Roullet, 1994) and are less likely to habituate to a novel situation. This higher level of emotionality of BALB mice could account for the lower base activity level of the BALB-derived RI mice in the present study, because fearful mice may be more likely to freeze on the beam or to hide in the confines of the nest box during wheel testing. It is therefore not surprising that strain differences were found in the present study. It is important to note that the size of this strain difference was larger than differences due to CC abnormality, because CC transected B6D2F_2 mice generally outperformed even RI mice with intact CC.

There is no previous research on split brain mice which could be compared with the present set of experiments. However, because the pattern of callosal projections in the mouse is highly similar to that in the rat (Olavarria and van Sluyters, 1995), interspecies comparisons can be made with only minor reservations. Using a two-choice visual discrimination task, Christie and Russell (1990) established different running patterns in rats which depended on the side of approach to the apparatus. After CC split surgery, the trained rats performed at chance levels when using the eye ipsilateral to the preferred side of approach, due to disruption of the stereotyped running pattern which had been established prior to CC transection. CC transected rats no longer adapted their pattern of movement to the visual input, which indicates that CC section interfered with visuomotor integration. By contrast, Noonan and Axelrod (1991) found that CC transected rats were superior to controls in the acquisition of a left-right response differentiation on a water maze task. This advantage of split brain subjects was greatest when the CC in its entirety was split, intermediate after anterior or posterior CC transections and did not appear after transections of the central CC (Noonan and Axelrod, 1991). These findings clearly implicate the CC in left-right confusion in normal rats; the effect appears to be dependent on transcallosal transfer of both motor information in the anterior CC and sensory information in the posterior CC.

The CC is also involved in the transfer of motor information during place and landmark navigation tasks. Split brain rats with unilateral lesions of parietal cortex exhibited severe deficits on spatial navigation, indicating that no motor information transfer from the intact hemisphere is possible (Crowne, Novotny, Maier, and Vitols, 1992). In addition, anterior CC transection has been shown to abolish the contralateral transfer of forelimb seizures in response to kindling in the hippocampus (McIntyre, Stokes, and Edson, 1986). At least one piece of research indicates that the callosal split mainly affects motor behaviours that are dependent on head movements (Sullivan, Parker, and Szechtman, 1993). In that study, a preferential direction of dodge from an attacker was not abolished by CC transection, but an asymmetry in the direction of edge investigation (mainly consisting of sniffing and head movements) was eliminated by CC split surgery. Clearly, past research has demonstrated that in rats motor behaviour is disrupted by CC transection on tasks that are dependent on head movements and lateralized responses. The present study is the first to report impairments of motor coordination on a highly complex but non-lateralized task in split brain mice. Mice could run on the running wheel in either direction, although the presence or strength of a preferred side of approach to the wheel was not measured. It remains to be investigated whether forcing a unilateral approach to the wheel running task would exacerbate the deficit in either CC transected or acallosal mice.

Research on laterality preferences on motor tasks is abundant in the literature on acallosal mice. The majority of studies found no evidence of a relationship between CC size and paw preference in BALB mice (Schmidt, Manhaes, and de Moraes, 1991; Ward, Tremblay, and Lassonde, 1987; Bulman-Fleming, Wainwright, and Collins, 1992), although one study showed that male mice of the I/LnJ strain (which is always acallosal) have a reduced strength of paw preference. More recently, stronger evidence of callosal involvement in motor behaviours of acallosal mice has been reported (Nakatsuka, Watanabe, Murakami, Negi, Itano, and Shimada, 1994). In the latter study, the motor cortex in a large sample of ddN mice (which have a low incidence of callosal agenesis) was electrically stimulated, resulting in contralateral forelimb contraction in ddN mice with normal CC. By contrast, acallosal ddN mice exhibited not contralateral but ipsilateral forelimb contraction. This finding clearly illustrates not only a lack of contralateral transfer of the stimulus, but also the use of an abnormal ipsilateral projection in the acallosal mice.

All of the above studies serve well to demonstrate that in acallosal mice there is no or reduced contralateral transfer of motor information and that there may be abnormal ipsilateral transfer. One further study found that this lack of transfer can result in impairments on motor coordination. Lipp, Wolfer, Waanders, and Magara (1990) tested samples of mice from acallosal strains with incomplete penetrance of the defect on a battery of behavioural tests and found a clear correlation between callosal size and performance on sensorimotor tasks including hindlimb coordination on a balance beam. In particular, acallosal mice exhibited a high variability of performance levels compared to normal littermates which, however, was not replicated in the present program of research.

Literature on the lack of transfer of motor commands or motor skills in acallosal or split brain humans is equally abundant. Lassonde, Sauerwein, and Lepore, 1995), for example, demonstrated that both groups are impaired on tasks requiring the transfer of visuomotor information or a motor skill. On a task requiring key presses with either hand in response to visual stimuli, acallosal, split brain and control subjects all had superior reaction times if the stimulus and response key were on the same side of the midline and if the hands were not crossed during responding. The two CC deficient groups, however, exhibited deficits relative to subjects with normal CC, whenever the task demands involved interhemispheric transfer of visuomotor information or integration of sensory information across the midline.

The ability of acallosal and split brain subjects to use ipsilateral motor control can be assessed in tests which require patients to control proximal and distal musculature to reach into the visual space contralateral to the hand they are using. Jacobson, Servos, Goodale, and Lassonde (1994), demonstrated that acallosal subjects are generally slower when reaching with either hand across the body midline and are also impaired at grip formation with hands reaching across the midline. This indicated an impairment in the control of both distal and proximal upper limb musculature. Later research confirmed this finding for both acallosal and split brain subjects and indicated that similar deficits are not found for the control of lower limb or axial musculature when reaching across the midline, which is controlled via bilateral motor pathways (Berlucchi, Aglioti, Marzi, and Tassinari, 1995).

Ultimately, what do the results of the present study indicate with regard to compensatory mechanisms for ACC? As mentioned earlier, little support exists for a bilateral representation of functions in ACC, and our results, which indicate a clear disadvantage in cases of ACC, lend no support whatever. Cross-cueing is equally unlikely, and the acallosal RI mice in our colony have never exhibited any strange or unusual behaviours indicative of cross-cueing during behavioural testing by experienced observers. The most likely possible compensatory mechanisms therefore remain the use of other commissures and/or the use of uncrossed projections. While much well-learned, automatic motor behaviour is coordinated by spinal circuits or at the level of the cerebellum, and consequently is independent of callosal transfer, higher processing evidently is necessary for some aspects of the complex bimanual tasks employed in the present set of behavioural tasks.

The use of extracallosal commissures is neither contradicted nor supported by our data. Obviously, using commissures such as the AC for complete interhemispheric transfer of information usually carried by callosal fibres would result in no or very minor deficits, or even an improvement compared to normals. Whether any deficits or gains would be observed at all would depend on the number and capacity of these extracallosal fibres, and on their relative length and speed of conduction. Regardless of whether extracallosal commissures were employed in our acallosal subjects, it appears clear that the size of their deficit cannot be explained by this compensatory mechanism alone. However, the use of extracallosal commissures may be combined with the use of ipsilateral projections which have not undergone postnatal degeneration, or which may even have expanded, in cases of ACC. As previously mentioned, these projections may carry insufficiently detailed information that may result in incorrect responses on motor tasks requiring very precise motor output.

Considering the incredible anatomical and functional plasticity the brain possesses, especially in cases of congenital disorders, it appears unlikely that only a single compensatory mechanism would be employed for any defect with consequences as far-reaching as those of a lack of the major cerebral commissure. Probably, both extracallosal commissural fibres and ipsilateral projections are employed, and it appears evident that when task demands are sufficiently high, even this much is not enough.

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Chapter 5

GENERAL DISCUSSION

The program of research described in the present document was undertaken in an attempt to elucidate mechanisms of functional compensation for the congenital absence of the CC, the major commissure connecting the cerebral hemispheres. It has long been a matter of great interest to researchers in this field of study that patients affected by ACC do not display the familiar split brain syndrome exhibited by patients who have undergone surgical transection of the CC in adulthood. The explanation for this lack of symptoms must lie in behavioural or anatomical compensation for the congenital defect. This compensation, due to a comparative lack of neural plasticity, can not occur to the same degree in adult patients who have undergone callosotomy surgery.

The most interesting feature of the pattern of behavioural deficits after surgery as compared to ACC clearly lies in the fact that whereas congenitally acallosal subjects do not exhibit the split brain syndrome, they do appear to have selective deficits on bimanual coordination tasks. These deficits, which are exacerbated in conditions of high task demand, are not observed in split brain patients. This problem with bimanual coordination indicates that there must be some aspect of the functional and neural compensation for ACC which actively interferes with bimanual motor coordination, causing deficits greater than those observed in split brain patients in whom there is little remaining interhemispheric integration of motor function.

Two plausible routes of neural compensation for ACC have been suggested and thus far not been contradicted by the available evidence. One compensatory mechanism may be that alternate routes of interhemispheric transfer of information, such as the AC, are utilized to a greater extent in cases of ACC. The alternate explanation for compensation is that subjects with ACC may use ipsilateral projections, such as extralemniscal sensory pathways, which in the normal nervous system either degenerate after birth, or which are inhibited by contralateral projections or the CC itself. In ACC subjects, these fibres could enable both hemispheres to gain access to information which is restricted to the hemisphere of input in split brain patients. Competing information carried in ipsilateral and contralateral sensory fibres might then result in poor stimulus topography. Less precise motor control exerted via ipsilateral motor fibres might result in clumsy movements.

These two possibilities for neural compensation were tested with a series of behavioural tests which compared normal, congenitally acallosal, and split brain mice with respect to bimanual motor coordination under conditions of high task demand designed to exacerbate any possible behavioural deficits. Appropriate anaesthetic and surgical methodology was developed to permit the use of large sample sizes of split brain mice, the only nonhuman species known to exhibit the congenital deficit. If extracallosal commissures or subcortical interhemispheric projections are used as compensatory routes in ACC, mice with the congenital defect should have a relative advantage over callosotomized mice. In that case, normal mice should perform best at tasks requiring interhemispheric coordination of movement because they could utilize the CC. Acallosal mice should perform at an intermediate level, because some information could be transferred interhemispherically via alternate routes. Split brain mice, which presumably can transfer little or no motor information between the hemispheres, should perform worst. If, on the other hand, ipsilateral fibres are used to compensate for the congenital deficit, acallosal mice would perform worse than split brain mice, if the control exerted by these fibres would interfere with behaviour exhibited in the absence of interhemispheric communication by callosotomized mice. Neurologically normal mice, of course, would still be expected to perform best.

Initial research was concerned with the development of methods for anaesthesia and callosotomy surgery in mice. Due to their small size, mice are rarely chosen for surgical treatment; however, due to the fact that they are the only nonhuman placental species to exhibit the congenital defect, their use in this study was imperative. The first problem working with various strains of mice was the fact that there are both large interspecies and individual differences in susceptibility to anaesthetic agents. The use of isoflurane gas, whose dosage can be adjusted easily and quickly, resulted in far lower fatality rates due to overdosing during anaesthesia. Overall time required for surgical induction was also significantly reduced, and in case of surgical complications the duration of anaesthesia could be extended without the risk of fatality associated with supplemental injections of injectable anaesthetics. These advantages, in my opinion, far outweigh the disadvantage of initially higher cost for anaesthetic machinery and anaesthetic agent itself.

The surgical method for callosotomy in mice mainly addressed the problem of precision microsurgery of small structures in the brain. At the midplane, the mouse CC is less than 1 mm thick, and rarely above 4 mm in length (personal observation). Most common surgical procedures are relatively imprecise and would have resulted in significant extracallosal damage . The procedure developed in the present experiment differs in three main respects from more common methods. General precision of all measurements was increased by a factor of 10 to 50. All distances were measured to 0.01 mm, and angles were measured to 0.1° precision, instead of measuring to 0.1 mm and 5°, as is commonly done in experimental rodent brain surgery. In addition, a three cut approach was taken to transecting the CC. Any single straight line cut through the CC would necessarily have resulted in significant extracallosal damage along the path of entry. By contrast, damage due to this three cut approach to surgery was restricted to the CC, dorsal fornix, and a small area of cingular cortex, whose role in sensorimotor processing is not known (Koralek, Olavarria and Killackey, 1990), but which is implicated in the learning of conditioned responses (Freeman, Weible, Rossi, and Gabriel, 1997). Lastly, the CC was not transected by inserting a knife forward through the structure. The micro knife used during surgery was very thin and relatively flexible. As a result, the tip of the knife would have been deflected by the dense bundle of transverse fibres constituting the CC. Instead, the knife was inserted just dorsal to the CC, then deflected ventrally by changing the relative angle between the knife and the CC, and the callosal fibres were transected by the hook-shaped end of the knife during withdrawal of the instrument. As a result of these methodological changes, the CC was reliably transected with minimal extracallosal damage.

A further preliminary study focussed on the gross anatomy and development of the acallosal mouse brain. Several different recombinant inbred mouse strains with various degrees of callosal agenesis were used in the present set of studies and compared to hybrid mice with or without transected CC. Although some researchers have found no changes in locomotor activity with inbreeding of mice (Deckard, Wilson, and Schlesinger, 1989), mutations of even single genes can affect complex motor behaviours in mice (Olivero and Messeri, 1973). BALB/c mice, one of the parent strains used to breed the RI strains in the present series of experiments, are more timid than hybrid mice and lack the infrapyramidal mossy fibre layer of the hippocampus (Lassalle, Halley, and Roullet, 1994). When using strains of mice of varied genetic background, behavioural differences not due to influences of callosal status might therefore be found. This concern was partly addressed in the present set of experiments, because the two groups of mice with normal callosa were composed both of normal hybrid animals, and of normal RI mice from strains derived from the same ancestors as the acallosal RI mice. Some of the strains used actually exhibited incomplete penetrance of the callosal defect, which meant that different mice from the same litters were included in the acallosal group or in the normal group and therefore the effects of having a normal versus an absent CC could be compared both between and within RI strains. Normal mice could be of the same genetic background as surgically treated B6D2F_2 subjects or as congenitally acallosal RI subjects; thus, there was no systematic genetic difference between normal mice and mice from other groups.

By contrast, there were systematic genetic differences between surgery subjects, all of which were derived from hybrid mice, and acallosal mice, all of which were derived from various RI strains. It was therefore important to determine whether there are behaviourally significant differences in neural development between these strains. Overall brain development in various strains of mice is related to development of the cerebral cortex, which is associated with higher cognitive functions and consciousness. Because areas of the cortex are interconnected via the CC, the congenital absence of the CC could interfere with normal cortical development and thus with normal behaviour. The thickness of various areas of the cortex was therefore assessed in order to compare general development of cortex in the strains used in behavioural tests. Generally, cortical thickness was not affected by callosal status but depended most clearly on overall size of the subject. However, thickness of the medial and cingulate cortex was affected by callosal development. These areas of the cortex were actually thicker in mice with smaller or missing callosa. If this increase in cortical development did affect behaviour in acallosal mice, however, it should have a performance-enhancing effect. Because acallosal mice had deficits on behavioural tests, as compared to performance by normal mice, it is unlikely that these behavioural differences were caused by the changes in gross cortical development associated with ACC. In addition, although acallosal mice performed worse than split brain mice and normal RI mice, differences were found not just in the overall level of performance, but in the pattern of performance: both normal RI mice and CC transected hybrids showed improvements in performance over time, whereas congenitally acallosal mice appeared incapable of significant improvement.

The remainder of the experiments described in this thesis focussed on the behaviour of normal and CC transected hybrid mice as well as RI mice with normal, partially defective, or absent CC. Two paradigms for testing bimanual motor coordination were used, running on a wheel and crossing a narrow balance beam. Neither test used reinforcement or punishment to evoke motor output, but rather relied on intrinsic motivation. In addition, both tests had high task demands, using conditions that required fine coordination for movement across discontinuous surfaces.

The first test used a running wheel. In past research, mice and other rodents have often been motivated to run on wheels by food deprivation and there is indeed a clear link between food deprivation and increases in activity on a running wheel (Routtenberg and Kuznesof, 1967; Epling and Pierce, 1984). However, tests of mice on running wheels have shown that mice will run several kilometres per day even if not food deprived (Bishop, Kruyer, and Wahlsten, 1996), indicating that wheel running is an intrinsically rewarding behaviour in mice.

There were several major findings from this experiment. Overall, hybrid mice performed superior to RI mice, and females were somewhat superior to males. Mice with CC defects performed at lower levels than mice with normal CC, regardless of strain. Hybrid mice with CC transection generally displayed better performance than mice with ACC but had fewer very long bouts of running than normal hybrid or RI mice. The deficits in acallosal RI mice were not only greater than those in any other group but also very persistent, and this was the only group which displayed no improvement in performance over days.

The second test of bimanual coordination employed a narrow balance beam which mice were free to traverse between nest boxes and platforms. Again, no external motivation was offered to subjects to perform. Results of the balance beam test closely parallelled findings on the running wheel. Again, hybrid mice overall performed better than RI mice, but the performance levels were lowest in the congenitally acallosal group, in which more slips and falls off the beam were observed than in all other groups. Thus, when using the above two bimanual tasks with high task difficulty, any neural compensation for the lack of interhemispheric integration in the acallosal group actually interfered with performance relative to behaviour displayed by mice with recent surgery to transect the CC, which are not capable of neural compensation for the lack of interhemispheric fibres by growth of new neurons or regeneration of transected axons (although some compensation would be possible via the formation of new synaptic connections).

The third behavioural test employed here differed substantially from the other two tests in that it employed a highly overlearned, unimanual task. Claw cutting is displayed by about 80% of rats as part of their grooming routine (Whishaw, Kolb, Sutherland, and Becker, 1983), and involves biting off the claw with the incisors. Lifting a hind paw to the animal's rostrum for cutting requires general motor coordination in order to keep the animal balanced during claw cutting. Claw cutting itself is controlled by the primary motor cortex, as shown by the fact that the behaviour is abolished by frontal decortication (Whishaw et al, 1983). The present research indicated that CC abnormalities do not abolish the behaviour. Hybrid mice, regardless of CC status, had shorter claws, indicating proficiency at claw cutting. RI mice had longer claws than hybrids, indicating a deficit on this behaviour. The only influence of CC status on claw cutting was the fact that normal RI mice improved at the task with age and increasing experience, whereas acallosal RI subjects did not. The relative lack of effects may have been due to the low task difficulty and the fact that only length of claws was scored rather than the quality of the cut. In fact, if a claw was not cut for a very long time, it might conceivably tear or break off, resulting in very short claw length, but a ragged edge.

In reviewing the behavioural evidence presented in Chapter 4, there emerges a definite pattern of relative motor impairments in the different groups. Overall, the performance of control mice, regardless of strain, was best, callosotomized mice performed at an intermediate level, and acallosal mice performed worse than all other groups. In addition, on all behavioural tests hybrid B6D2F_2 mice were superior to recombinant inbred strains of mice. One major difference between RI mice with intact CC and those suffering from ACC was that, in spite of initial deficits, those with intact CC were capable of improvement on behavioural tasks, while those with ACC were not.

One factor which could account for the deficits in bimanual coordination seen in both CC transected and acallosal groups would be a general deficit in somatosensory perception. Clearly, if the subjects had insufficient somatosensory integration to detect the relative positions of limbs, coordination would be compromised even in the absence of any motor deficits. There is limited evidence of somatosensory discrimination deficits in both ACC and split brain patients. In particular, these deficits affect only discrimination of axial somatosensation, while sensation in limbs and hands appears unaffected (Schiavetto, Lepore, Lassonde, 1993). Split brain patients, who have little or no neural compensation for the lack of the CC, can even transfer visual information (Sergent, 1991). However, only simple binary information appears to be shared between hemispheres, and this could be transferred via posterior or collicular commissures (Corballis, 1994), or even via cross-cueing.

By contrast, there is abundant evidence that a lack of callosal fibres is related to deficits in motor integration and coordination. It has been shown that in situations where unilateral visual stimuli are presented, information transferred via the CC is not sensory in nature. Rather, visual information is processed unilaterally and premotor information is then transferred to the contralateral hemisphere via the CC (Iacoboni, Fried, and Zaidel, 1994). However, exclusive transfer of motor information is not intuitively appealing, because in some cases information may need to be available to both hemispheres even if the behavioural output does not require gross movement. Verbal responses to visual or somatosensory stimulation presented to the hemisphere which is not dominant for language processing, for example, would require interhemispheric transfer of the sensory information. Bimanual coordination, on the other hand, would require the transfer of motor information. Anatomical evidence supports the contention that the type of information transferred interhemispherically via the CC is primarily motor information. Motor fibres course mainly through central and posterior parts of the CC and there is a visible decrease in thickness of the body and splenium of the callosum after deafferentation in diplegic patients (Iai, Tanabe, Goto, Sugita, and Niimi, 1994). In addition, there is no evidence to support significant transfer of motor information via extracallosal commissures in neurologically normal subjects, whereas visual information can be transferred via the intertectal and posterior commissures (Corballis, 1994). Therefore, callosal defects would result in deficits only on those tasks which require the transfer of motor information.

The factor which determines which type of information, sensory or premotor, is transferred, may depend on stimulus characteristics and task requirements (Bisiacchi, Marzi, Nicoletti, Carma, Mucignat, and Tomaiuolo, 1994). This may account for the fact that some deficits in acallosal patients are severe, while function appears unimpaired on other tasks. When the information which needs to be transferred between the hemispheres is purely sensory, extracallosal commissures can be utilized for transfer. For gross, overlearned movements, there may be no need for callosal transfer, because these could be controlled by the cerebellum (Geffen, Jones, and Geffen, 1994). When interhemispheric transfer is required for control of precise movements, the only possible alternate route of interhemispheric transfer would be via the AC.

The major problem with the latter suggestion lies in the fact that no non-callosal mechanism for interhemispheric transfer between frontal and posterior parietal areas has ever been described (Lassonde, 1994; Risse, LeDoux, Springer, Wilson, and Gazzaniga, 1978). In one study, the retrograde tracer horseradish peroxidase (HRP) was injected into the rat AC in order to trace the cells of origin of this commissure (Jouandet and Hartenstein, 1983). Labelling was observed in the pyramidal cell layer of perirhinal cortex and neocortex ventral to the rhinal sulcus. No labelling was found in more dorsal parts of the neocortex, where limb control is localized. Another study, in which the fluorescent tracer DiI was used to trace interhemispheric connections via the AC also failed to find any labelling in the frontal motor cortex, but indicated projections to the pyriform, insular, temporal, perirhinal, and entorhinal cortices (Lent and Guimaraes, 1990). Further evidence against the possible use of the AC in cases of callosal agenesis comes from a detailed study of AC anatomy in acallosal mice which demonstrated that there is no increase in AC diameter in acallosal mice (Livy, Schalomon, Roy, Zacharias, Pimenta, Lent, and Wahlsten, 1997). Only unmyelinated AC fibres were somewhat increased in number in acallosal mice, providing some anatomical compensation for the callosal defect. In view of the relatively minor increase in the number of slow, unmyelinated AC fibres, it remains unlikely that much functional compensation for ACC can occur via the AC of affected individuals. Whatever compensation there is, however, would not be involved in the transfer of motor information.

The only plausible alternative is that ipsilateral and contralateral motor projections controlled by the same hemisphere must be used to control bilateral coordination of the limbs. If the control exerted via the ipsilateral connections is inferior to or interferes with that exerted via the contralateral motor projections, coordination of movements would necessarily be impaired, and this is precisely what the present series of studies has confirmed. Evidence shows that in the normal brain, the CC is responsible for synchronization of bilateral responses and minimization of CUDs (crossed uncrossed differences, the difference in time required for responses when stimulus and response are processed in the same hemisphere as compared to different hemispheres). In brains with defects of the CC, whether acquired or congenital, CUDs are indeed larger, though there still is some synchrony of bilateral movements (Berlucchi, Aglioti, and Tassinari, 1994). This means that in acallosal and even in split brain subjects, there must be a mechanism for access to motor information about the entire body or for motor control over both sides of the body within one hemisphere of the brain. Electrophysiological evidence has shown that this can occur via ipsilateral motor control (Nakatsuka, Watanabe, Murakami, Negi, Itano, and Shimada, 1994), because both normal and acallosal ddN mice receiving electrical stimulation to portions of the motor cortex while anaesthetized exhibited forelimb contraction. However, in neurologically normal mice the contralateral forelimb responded, whereas in acallosal mice, the response was also seen in the ipsilateral forelimb. This constitutes clear evidence for the existence of ipsilateral motor control in acallosal mice, most likely as a result of a failure of early ipsilateral projections to degenerate in cases of ACC where no interhemispheric transfer of motor information is possible. In addition, the response latency in the acallosal group was actually shorter than that in normals. Apparently, transmission time in the acallosal group was reduced, because the distance from the cortical stimulus to the responding limb was shorter in the ipsilateral response where no interhemispheric transmission was involved.

The most likely substrate for ipsilateral motor control is the corticoreticulospinal tract. Research using rats has indicated that this tract can be used for ipsilateral motor control in the normal brain. However, it was suggested that in the normal brain, the contralateral and ipsilateral corticoreticulospinal tracts inhibit each other, permitting sole control of contralateral motor responses via the pyramidal tract (Vahlsing and Feringa, 1980). This would mean that the ipsilateral projections exist not only in acallosal brains, where plastic compensatory changes in the early developmental period would prevent degeneration of ipsilateral projections, but that it also exists in normal brains, where it is inhibited by the contralateral corticoreticulospinal tract via the CC. Some recovery of ipsilateral control would thus still be possible in split brain patients, although it is unlikely that this recovery would permit the same degree of ipsilateral control as in acallosal brains.

There are in fact several pieces of experimental evidence supporting the existence and use of ipsilateral motor projections in split brain subjects. Lehman (1968) worked with macaques undergoing staged CC transections and unilateral optic tract transection in the hemisphere contralateral to the preferred hand. Subjects with intact posterior sections of the CC were capable of fine grasp of visually presented stimuli with either hand, but they reversed their hand preference, using the hand contralateral to the intact optic tract. Subjects with complete CC transection showed similar behaviour, demonstrating that in both groups, visual information could be transferred contralaterally via the posterior section of the CC or via extracallosal pathways. However, when the subjects with complete CC transections were forced to use the hand ipsilateral to the intact optic tract, movements were clumsy and groping. Thus, ipsilateral control could be forced but was imprecise. The low quality of ipsilateral control in these subjects was similar to what is seen in acallosal patients who perform tasks requiring bilateral coordination, which can be achieved only if both hands are controlled by the same hemisphere.

There is also some behavioural evidence for the use of ipsilateral projections in human split brain patients. Patients with transections of the CC sparing the posterior CC showed negative CUDs for key presses in response to unilaterally presented visual stimuli. These patients actually had faster reaction times when using the hand ipsilateral to the hemisphere processing the visual stimulus, which could be explained by the use of ipsilateral motor projections without the need for interhemispheric transfer of information. There is evidence that split brain patients are capable of ipsilateral control of fingers, even when cross-cueing is prevented (Trope, Fishman, Gur, Sussman, and Gur, 1987). Two human patients with chronic split brain surgery were tachistoscopically presented with images of hands on which one finger was marked. The patients performed best at moving the indicated finger when using the hand contralateral to the hemisphere processing the visual stimulus. When ipsilateral control was forced, the patients could control the thumb and index finger but not the remaining fingers. This again constitutes behavioural evidence for ipsilateral motor control in split brain patients; however, this behaviour had to be forced and control was incomplete.

The use of ipsilateral projections in split brain patients therefore appears to be insufficient for full control of movements. It is likely that in acallosal subjects, ipsilateral control is more fully developed, due to the fact that the neural defect is of a congenital nature. This means that neural compensation for the defect occurs early in life, when functional and anatomical plasticity is at a maximum (Schneider, 1987). The evidence cited above demonstrates that split brain patients can be forced to use ipsilateral control in unimanual tasks, but there is no evidence of ipsilateral control when it is not essential for performing a task, or when bilateral coordination is required. On the other hand, the only possible mechanism which can account for the fact that acallosal subjects have greater deficits on tasks requiring bimanual integration than split brain subjects in spite of the fact that they have significantly greater opportunity for neural compensation, is that acallosals do use ipsilateral projections even for bimanual tasks which do not explicitly require ipsilateral control.

The precise reasons why an increased reliance on ipsilateral control may interfere with performance on bimanual tasks are still unclear. One plausible suggestion is that ipsilateral projections carry imprecise information, that the control exerted via ipsilateral motor fibres is in itself inferior to that exerted by contralateral fibres. Another, somewhat more cogent explanation is that there is interference between ipsilateral and contralateral fibres controlling the same limb of acallosal subjects. When performing a bimanual task, acallosal as well as split brain subjects process sensory information independently in each hemisphere. In split brain subjects, each hemisphere then independently controls the contralateral limbs. This leads to some incoordination between limbs and some deficits on performance. When performing the same bimanual task, acallosal subjects rely more strongly on ipsilateral motor projections. Therefore, each hemisphere independently processes sensory input and independently controls and coordinates movement of both the contralateral and ipsilateral limbs. Each limb therefore receives motor afferents and competing motor commands from both hemispheres. This leads to even greater problems in motor coordination and in precise motor control than is found in split brain subjects, in which each limb receives motor afferents from only one source. Due to the fact that both hemispheres independently receive visual feedback about overall motor performance and the relative positions of both limbs, motor commands from both hemispheres tend to correspond, leading to satisfactory motor coordination on all but very complex tasks requiring precision movements, or tasks performed without visual feedback.

Obviously the present series of experiments offers better evidence for the use of ipsilateral motor projection in acallosal mice than was previously available, mainly due to the fact that large samples of mice were tested under controlled testing conditions, whereas most previous evidence was anecdotal in nature or at best based on case studies of small samples of human subjects. The present set of experiments also constitutes the first systematic comparison of bimanual coordination in large samples of acallosal and split brain subjects. Equally obviously, some questions remain unanswered. It would be desirable to have further information on the comparative abilities of the different strains used in this study. Although there was no gross abnormality in cortical development of acallosal subjects, overall cognitive abilities were never assessed. General memory could be compared between strains in matching-to-sample tasks, which would give some insight into learning abilities of the strains. The motor tasks employed here did not require high cognitive abilities; however, memory deficits might account for at least some of the lack of improvement with practice seen in the acallosal mice.

Another intriguing avenue of investigation would be to test whether any bimanual task could be overlearned to the point where reliance on callosal transmission disappears with practice as motor control shifts from cortex and transcallosal integration to cerebellar control. If this was the case, the deficits observed in split brain and acallosal subjects should disappear with practice. Preliminary evidence from the claw cutting task does indicate that such improvement might not occur in the acallosal subjects, because adult ACC subjects had not acquired the normal skill at this task during their lifetime. If this finding could be confirmed, it could indicate that the congenital absence of the CC is associated with cerebellar defects, which could further account for the clumsy motor output characteristic of acallosal subjects. This possibility is unlikely, however, in light of the fact that general motor coordination on overlearned bimanual tasks with low task demands, such as walking, has never been reported in the literature on human ACC subjects and has not been observed in casual contact with acallosal mouse strains (personal observation). The question of whether ACC mice have deficits on overlearned movements such as walking might be tested in future research by measuring the regularity of footprint patterns. Alternatively, the neural compensation which occurs in the congenital condition somehow might interfere with the shift of control seen in normal subjects and thus result in the relatively permanent nature of motor deficits, even after extensive practice.

A number of problems with normal motor function clearly are part of the clinical picture in human and murine acallosals. There is a lower extent of bimanual coordination than is possible in split brain subjects, probably due to competing motor information carried to each limb in afferents from both the ipsilateral and contralateral cerebral hemispheres. There is the possibility of problems in shifting overlearned movements to cerebellar control. There are also surprisingly few problems on everyday motor abilities. Knowledge about the neuroanatomical mechanisms of these problems and about the reason for the lack of greater problems could provide valuable insights. This knowledge could help determine how much neural plasticity really is possible in congenital conditions and whether any of the compensatory mechanisms could still be developed with training or stimulation in the case of neurological defects acquired later in life. If ipsilateral motor projections still exist in the normal adult brain, could they be made available for use in patients suffering from stroke-induced or accidental cortical lesions by removing inhibitory projections? In the long term, this research will not only give insight into the abilities and deficits of rare acallosal patients, but it could even give hope toward the development of new avenues of treatment of hemiplegic patients. Finding and fully activating ipsilateral motor control fibres could give these patients the ability to perform normally at least on those tasks with low task demands such as walking. And after all, wouldn't that be the single most important function to regain?

5.1. Bibliography

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Appendix 1

AN INEXPENSIVE, LOW FLOW RATE, OPEN SYSTEM FOR ISOFLURANE ANAESTHESIA IN MICE

A major problem in anaesthesia for small rodent surgery is the great variability within and between strains regarding susceptibility to the anaesthetic (Fujii, Hara, Meng, Vonsattel, Huang, and Moskowitz, 1997; McCrae, Gallaher, Winter, and Firestone, 1993). With injectable anaesthetics, dosage cannot be downregulated once it is administered and supplemental injections to increase depth or duration of anaesthesia may result in high rates of mortality.

With the development of fluorinated ethers in the 1940s a range of new, relatively safe gas anaesthetics became available, including enflurane, halothane, and isoflurane (Wade and Stevens, 1981). Both halothane and isoflurane are very potent and volatile (Paddleford, 1992) and have similar vapour pressures at room temperature. Thus, they can be used in the same vaporizer after reconditioning by the manufacturer (Haskins, 1992). Both of these anaesthetics have relatively low blood solubility, which contributes to rapid recovery (Evans, 1992). However, isoflurane has added advantages, one of which is the fact that the difference between the anaesthetic dose and lethal dose in rats is twice that of halothane (Kissin, Morgan, and Smith, 1983). In addition, isoflurane anaesthesia results in lower myocardial depression (Haskins, 1992) and provides global cerebral protection against hypoxia during neurosurgery, because both cerebral metabolism and cortical electrical activity are depressed by isoflurane (Newberg and Michenfelder, 1983).

The main drawback of isoflurane anaesthesia is its relatively high cost, which includes the initial purchase of the anaesthetic and scavenging apparatuses and the ongoing expense of the anaesthetic itself. These monetary considerations were addressed by developing a technique which utilized a vaporizer, a simple hose attached to the room air exhaust in place of scavenging equipment, and flow rates far below manufacturer's recommendations, which minimized anaesthetic waste.

A1.1. Materials and Method

A1.1.1. Apparatus

An open system gas anaesthesia machine with a Fluotec MK2 vaporizer calibrated for isoflurane gas (supplied by Associated Veterinary Services in Edmonton) was used. Medical grade oxygen flow rate could be regulated between 200-2000 ml/min and nominal gas concentrations could be adjusted to between 0% to 4% on a continuous scale. A disposable plastic Bain's Circuit hose of approximately 1.2 m length was used to supply gas to the animal during anaesthesia. Because the distance from the outlet of the gas hose and surrounding corrugated scavenger hose in the mask adaptor piece of the Bain's hose was only 4 cm, anaesthetic gas, at very low flow rates, was drawn back into the scavenging circuit before reaching the subject. Thus, the adaptor piece was removed and the corrugated part of the Bain's hose was not used. The outlet of the gas hose was fastened at one centimetre distance from the subject's nose by firmly taping it to the palate clamp holder (see Figure A1.1).

It must be stressed that the anaesthesia machine was not designed for very low flow rates. The Fluotec MK2 vaporizer delivers the calibrated nominal isoflurane

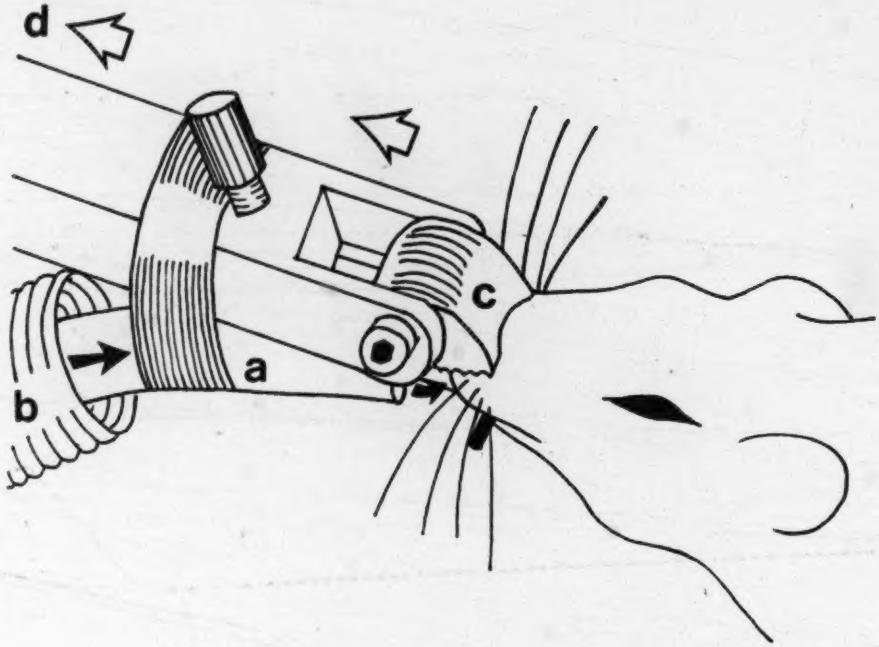

Figure A1.1. Lateral view of palate clamp used during mouse anaesthesia and surgery. (a) Isoflurane carrying part of Bain's hose, with (b) unused scavenging hose and (c) palate clamp used to fix subjects' heads. Solid arrows indicate direction of isoflurane gas flow and open arrows indicate direction of scavenge gas flow toward the intake of (d) scavenging hose anterior to subjects' heads.

concentrations at flow rates of 1000 ml/min or above. At lower flow rates, the actual concentration of isoflurane is significantly lower than the nominal concentration indicated on the machine and decreases with flow rate. Actual isoflurane concentrations used in the present experiment were not measured, but can be estimated from Table A1.1.

For scavenging unused isoflurane gas, the exhaust fan present in the room's ceiling was utilized. A flexible clothes drier hose was fixed to the ceiling fan intake using a cardboard box fitted to the fan. The end of the scavenging hose was placed approximately 15 cm anterior to the subject's head such that excess gas flow was directed away from the subject and experimenter. The flow rate of the air exhaust was approximately 15 cubic feet per minute, which was sufficient to remove excess gas at a rate at which it could not be detected by odour in the room air even after prolonged use of the anaesthetic setup.

A1.1.2. Anaesthesia

One hundred and seven B6D2F2 mice aged between 48 and 75 days were injected IP with 0.1 ml of atropine sulfate (0.4 mg/ml) 30 minutes prior to induction of anaesthesia. The induction chamber consisted of a 400 ml screw-top glass jar with a diameter of approximately 9 cm. A steel egg-shaped tea infuser fastened to the jar lid contained some cotton gauze onto which approximately 2 ml of isoflurane had been applied. This quantity of isoflurane was sufficient to induce anaesthesia in three mice

Table A1.1. Actual and Nominal isoflurane gas concentrations at various flow rates (supplied by Associated Respiratory Services, Edmonton)

Flow rate (ml/minute)	300	300	400	500
Nominal concentration (%)	2.0	2.5	2.0	2.0
Actual concentration (%)	1.21	1.87	1.4	1.78

over a period of approximately one hour before more isoflurane had to be applied.

Subjects were individually placed into the induction chamber and observed closely for onset of anaesthesia. After approximately 30 to 45 seconds, the subjects exhibited rapid, shallow breathing and an absence of voluntary movement. Approximately two thirds of all subjects temporarily assumed an ophistotonic posture characterized by an exaggerated stretch of the body with elevated tail and head and concave back. This lies within the range of incidence of ophistotonus observed previously in various mouse strains undergoing gas anaesthesia (Komatsu and Ogli, 1987).

As soon as this stage of induction was reached, the subject was removed from the chamber and its nose laid on the incisor bar of the palate clamp with isoflurane flow set at a flow rate of 500 ml/min at a nominal concentration of 4%. Within one minute, a slow, steady rate of breathing was generally observed, subjects had lost all apparent muscle tone and exhibited no corneal reflex. There was no pedal withdrawal reflex upon foot pinch, a commonly used method of gauging depth of anaesthesia (Park, Clegg, Harvey-Clark, and Hollenberg, 1992). At this time, the head could be fastened in the palate clamp and shaved, and isoflurane flow was lowered to 250 ml/min at a nominal concentration of 3% for females and 3.5% for males.

After completion of surgery, isoflurane gas flow from the anaesthesia machine was stopped, the palate clamp was loosened and subjects were injected IP with 0.5 ml/kg of buprenorphine (0.04 mg/ml) for postoperative analgesia. Mice were hand held until consciousness was regained and voluntary movement observed. This occurred in less than one minute in most subjects and never required more than three minutes even after unusually deep or prolonged anaesthesia.

A1.2. Results

Of 107 mice undergoing surgery (as described below and in Schalomon and Wahlsten, 1994) using this method , three died due to anaesthetic overdose during the induction phase, two never reached a sufficient depth of anaesthesia to proceed with surgery, and 102 were successfully anaesthetized and recovered from the combined effects of anaesthesia and surgery. Of these 102 subjects, one went into respiratory arrest within five minutes of being anaesthetized, and three more did so after being injected with buprenorphine. All of these subjects were revived by directing a stream of oxygen at their faces through the Bain hose (anaesthesia machine settings: 0% isoflurane, flow rate 1000 ml/min) while gently massaging the chest. They recovered uneventfully and were later used successfully in behavioural testing. Thus, the major complication with this method of anaesthesia was due not to the anaesthetic itself, but due to the administration of the postoperative analgesic, which appeared to delay the regaining of full consciousness by subjects, and to result in some respiratory depression. These complications were observed to the greatest degree if buprenorphine was administered immediately upon removal of the subject from the stereotaxic apparatus and thus from isoflurane gas. Both respiratory depression and effects on the level of consciousness were least severe if the subject was injected only after voluntary movement by the subject was observed.

As the volume of gas contained in the Bain hose between the anaesthesia machine and the subject is minimal (see also Haskins, 1992), amounting to approximately 60 ml, changes in concentration reached subjects within 15 seconds, and effects on anaesthetic depth could be observed within less than one minute. In most subjects, a steady plane of anaesthesia appropriate for a 30 to 45 minute surgical intervention was reached within two minutes at flow rates of 250 ml/min and concentrations of 2.5-3.0%. Individual variability in the hybrid mouse strain was high however, with females requiring on average less anaesthetic regardless of body weight and some individual subjects requiring as little as 2% at 200 ml/min or as much as 4% at 400 ml/min.

In order to test the flexibility of this method of anaesthesia further, several mice were anaesthetized very deeply for periods of up to 90 minutes. Under these conditions, one of four mice developed severe respiratory distress within ten minutes of anaesthesia onset. This was completely reversed within three minutes of downregulating isoflurane concentration and flow rate and did not result in death or postoperative complications.

A1.3. Discussion

Using the apparatus and methodology described here, fatalities due to anaesthesia can be almost eliminated. In addition, this system for isoflurane gas anaesthesia is, in the long run, cost effective for researchers performing small animal anaesthesia on a regular basis. The higher costs of initial purchase of apparatus and anaesthetic are offset by a reduction in time required for the surgery and by a significant decrease in the mortality of subjects.

Any significant respiratory depression which occurred after isoflurane use (similar to that previously described by Haskins, 1992, and Ludders, 1992) was probably exacerbated by the effects of the potent analgesic buprenorphine, which caused some of the postoperative complications described above. If compatible with local animal ethics regulations, it would therefore be advisable not to use any depressant analgesics in combination with isoflurane.

Another problem during induction is CNS disinhibition resulting in ophistotonus in mice, but not rats (Komatsu and Ogli, 1992). This phenomenon may be due to EEG spiking activity in frontal and parietal areas and is more pronounced with isoflurane than with other fluorinated ethers (Komatsu and Ogli, 1987). However, to date no long term problems due to ophistotonus have been reported and no relationship between ophistotonus and postoperative complications was apparent in the present set of surgeries.

Other health issues concerning the use of isoflurane have been raised. Although no systematic research on isoflurane carcinogenicity has been conducted in humans, one study, in which mice were exposed to low levels of the anaesthetic for 78 weeks on a daily basis, concluded that isoflurane is unlikely to have carcinogenic potential (Baden, Kundomal, Mazze, and Kosek, 1988). Similar exposure did not result in a decrease in fertility or increased reproductive wastage in mice (Mazze, 1985). Eger (1981) found that isoflurane biodegradation is significantly lower than that of enflurane or halothane, resulting in a relative absence of hepatotoxicity, nephrotoxicity, and pulmonary injury. In addition, administration of isoflurane, in contrast to that of enflurane and halothane, does not lead to myocardial depression, although the use of any of these gas anaesthetics may result in hypothermia (Eger, 1981). In the present study, this problem was addressed by placing the animals on a surface heated to body temperature during surgery. The use of a heat producing light source close to the subjects would likely be equally effective.

The advantages of isoflurane use are obvious. For researchers working with various strains of subjects or hybrid animals with a significant variability in drug susceptibility, the adaptability of dosage with isoflurane is invaluable. The preferred method of determining the required dose is to give a high dose initially, reducing this stepwise early in the procedure until a satisfactory depth of anaesthesia is attained (Dodman, 1992). If the surgical procedure is prolonged for any reason, no supplementary anaesthetic dose is necessary, thus eliminating one frequent source of complications of injectable anaesthetics. The major drawback of isoflurane, its cost, was addressed by the technique described here. The use of a reconditioned anaesthetic vaporizer and room air exhaust in lieu of a manufactured scavenging unit reduced start up costs significantly. In addition, the amount of anaesthetic gas actually required with an open system (one in which the subject is not intubated with the anaesthetic gas) is only about two to three times the subject's minute respiratory volume (Harvey, 1992).

Although the rate of gas flow we used was far below manufacturer's recommendations for the vaporizer, satisfactory results were obtained with very low flow rates simply by increasing nominal gas concentration, resulting in an affordable alternative method for anaesthesia in small rodents which entails a very low mortality risk.

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